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BIOLOGICAL BULLETIN

A CASE OF NORMAL IDENTICAL QUADRUPLETS IN THE NINE-BANDED ARMADILLO, AND ITS BEARING ON THE PROBLEMS OF IDENTICAL TWINS AND OF SEX DETERMINATION.[1]

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In a former contribution from this laboratory by A. M. Spurgin, M.D., occurs the following statement: "A year ago, Dr. W. M. Wheeler, of the School of Zoölogy, had the good fortune to secure four embryos of the *Dasypus novemcinctus* from an adult female which had been kept in the laboratory for several weeks. . . . He found four placentæ inclosed in one amnion, but has not since had time to study the subject further."

When the writers arrived in Austin last September they made arrangements to obtain material with which further to investigate this suggestive problem, and were fortunate enough to enlist the services of a naturalist living in a part of Texas where the armadillo abounds. In this way we have been able to secure several gravid females, from a study of which a number of interesting facts have been brought to light.

There are always four embryos, corresponding in number to the two pairs of mammæ, which are thoracic and abdominal in position. The embryos are not enclosed in one amnion, but each has its own distinct amniotic envelope, the cavity of which does not communicate with those of the adjacent amnia. This was proved by the experiment of inflating one amnion and noting that the air did not pass to the cavities of the contiguous amnia. The four amnia, however, are enclosed in a single chorionic vesicle.

[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 98.
[2] *American Journal of Anatomy*, Vol. III., No. 1.

An examination of the external villous layer of the chorion shows that placentation is unique in that areas of villi, although apparently forming a complete zone about the equatorial region of the uterine wall, are in reality arranged in four closely approximated ovoid areas, two large and lateral in position, and two smaller, one dorsal and the other ventral. The proximal (*i. e.*, the posterior or vaginal) and distal ends of the chorionic vesicle are practically free of villi, except at each pole, where the presence of a small tuft of villi causes the chorion to adhere very firmly to the uterine wall (see Fig. 1). The villous zone, composed as it is of four ovoid areas, extends into the polar regions in the form of four scallops at each end, and on the amniotic side of the four scallops are situated the points of attachment of the four umbilical cords (see Fig. 2).

In the advanced stages of gestation the chorionic vesicle is shaped very much like a football, and the four lines on the inside wall, along which the adjacent amnia meet, correspond to the seams of the ball (see Fig. 1). In the young stages, however, the chorion is in the shape of an octaedron with rounded edges, and flattened dorso-ventrally, and has its entire surface covered with villi (see Fig. 3). From this we may conclude that the definitive condition of the placenta has been attained by a localization of the villi into ovoid areas, each of which is to be looked upon as the vascular center for an umbilicus.

The points of attachment of the umbilical cords lie close to the amniotic partitions, and when the chorion is viewed from the distal end the cord in each case is seen to be situated just to the right of the partition (see Fig. 2). In embryos about one fourth grown the partition between any two contiguous embryos may be easily separated into its component parts, but in older stages the fusion of the membranes is so complete that separation is impossible. In late stages, therefore, the chorionic cavity is completely divided longitudinally into four separate compartments, each of which contains an embryo. Although the portions of the chorionic wall that lie between the successive amniotic partitions are in the form of quadrants, yet they do not coincide with the ovoid areas; for the partitions do not meet the chorionic wall in lines corresponding to the divisions of the ovoid

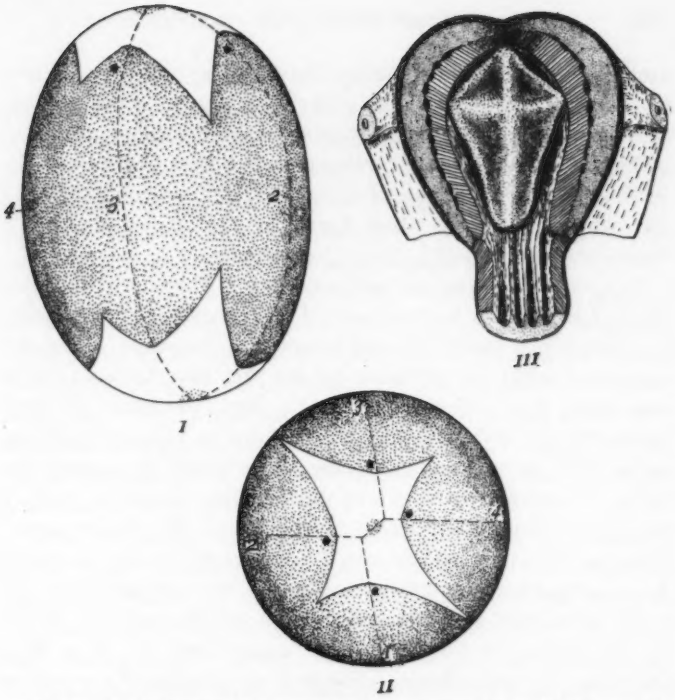

FIG. 1. A diagrammatic representation of an approximately dorsal view of a chorionic vesicle taken from the uterus about two weeks (estimated) before the young would have been born. Three of the ovoid areas are in view, and the broken lines represent the lines along which the amniotic partitions meet the inside wall of the chorion. The clear areas at the ends are broken into by the scallops of the ovoid areas. In this, as in the succeeding figure, the points of attachment of the umbilical cords are indicated by large dots. Note especially that the indentations between the scallops of areas 3 and 4 are much shallower than those between areas 2 and 3, leaving a broader connection between the former than between the latter. This is an indication that embryos 3 and 4, located respectively on dorsal and left lateral areas (similarly embryos 1 and 2, located on the ventral and right lateral areas), are natural pairs. For the significance of this arrangement see table and text. One half natural size.

FIG. 2. A view of the distal end of the preceding figure. This is introduced to show the relation existing between the amniotic partitions within the chorionic cavity. The figure also brings out the fact that the embryos may be paired, together with the ovoid areas to which they are attached by the umbilical cords. One half natural size.

FIG. 3. This shows a chorionic vesicle *in situ*, as revealed by splitting open the uterus along the mid-ventral line. The age of this vesicle is estimated at one month. Unfortunately the splitting was done before the specimen reached our hands, and extended so deep as to divide the vesicle into two parts. The parts, however, were well preserved *in situ*, and the reconstruction could be made with certainty. Note that the vesicle is octaedronal in shape, and that its entire surface is covered with villi. Natural size.

areas, but each partition passes through the middle of an area and thus intersects the apices of the proximal and distal scallops (see Fig. 1). The four partitions, furthermore, do not meet within the cavity in a line corresponding to the polar axis of the vesicle, but are fused in such a way that the cavities containing the embryos numbered 1 and 3 meet on their inner faces, while those containing 2 and 4 do not (see Fig. 2).

In a note in the current issue of *Science* (April 30) Professor H. H. Lane gives a brief account of placentation in the armadillo that differs in a number of particulars from the above. The discrepancies might be explained by the fact that Lane's account rests upon observations made upon a single deciduate placenta, taken after the birth of a litter. In addition to its incompleteness, his account differs from that offered here chiefly in locating the points of attachment of two of the umbilical cords on each of the large "disc-shaped areas," and none on the smaller areas. This condition is certainly not found in any of our material. A more fundamental difference between the two accounts lies in the interpretation of the condition of the chorion, Lane holding an opinion completely out of accord with the facts here presented, that the definitive chorion is the product of a complete fusion of four chorionic vesicles into one.

The unique interest of this case lies in the fact that the four embryos of a given set are not only invariably of the same sex, but are practically identical in many other respects. Measurements of the body and head lengths showed no essential differences between the individuals of a set of quadruplets, but a more searching comparison, consisting of carefully confirmed counts of the total number of plates in the nine bands of the armor, revealed, as one might expect, slight departures from complete identity. The results obtained from the examination of two sets will serve to indicate the small range of these departures.

Set A (Females).	Set B (Males).
No. 1.....556 (+1) plates[1]	No. 1.....571 (+2) plates
No. 2.....555 (+2) "	No. 2.....573 (+1) "
No. 3.....553 (+2) "	No. 3..... ..569 "
No. 4..... .....551 (+4) "	No. 4.....568 "

[1] The numbers enclosed in parentheses refer to certain rudimentary plates that are more or less united with other plates. It is impossible to tell in the embryos whether

In both cases the range of variation is five, or less than one per cent. The difference in totals between the two sets is very much greater — a difference that does not indicate a sexual dimorphism, for an examination of five unrelated specimens of both sexes that happen to be on hand, shows a range of from 547 to 575 plates, a variation nearly six times as great as that existing between a given set of quadruplets.

This very marked similarity among the individuals of a given set suggests that we have here a condition showing a degree of identity that, in all probability, is at least as close as that found in the well-known case of "true" or "identical twins" in the human race, where it is supposed that each embryo arises from from one of the two blastomeres of the two-cell stage. In the case of *Dasypus*, each embryo probably arises from one of the blastomeres of the four-cell stage.

This immediately turns our attention to a further consideration of the fœtal membranes, the amnion and the chorion, and of the manner in which they arise. It was stated above that each embryo is enclosed in a separate amnion, and that the four amnia are within a single chorion. We say single chorion advisedly, because its surface gives no indication of being a multiple structure, that is, the product of the fusion of four chorionic vesicles at an earlier period, as suggested by Lane; nor do the facts revealed by a study of sections of the chorionic wall seem to bear out such an interpretation. One must admit, however, the great difficulty of any attempt at a correct interpretation of the exact relationship existing between the fœtal membranes as seen in these advanced stages. The solution of that problem must be sought in a study of young stages, when the membranes are in process of formation.

To have four ova ripen, be fertilized, and reach the uterus at the same time, and always arrange themselves in a definite fashion

or not these small plates would later have become separated off to form distinct ones, but we are inclined to believe that such is the case, for in the adult armor there appear to be no signs of double plates. If we count these rudimentary plates as complete plates, then embryos 1 and 2 (Set A) would have exactly the same number of plates, as would also embryos 3 and 4. The numbers of plates in the corresponding embryos of Set B, although not identical, differ to the minimum extent, one plate in each case.

with reference to one another and to the uterine wall, would be in itself a remarkable coincidence. The chief difficulty, however, in the way of accepting the view that the chorionic vesicle is a multiple structure lies not so much in the interpretation of the relation existing between the fœtal membranes, nor in the discovery of the mechanics involved in the attainment of such a relation, as in the explanation of the fact that the embryos of any given set are always practically identical and of the same sex. At first sight it might seem that this identity could be explained by the fact that the embryos are apparently under the same environmental influences, inasmuch as they are enclosed within a single chorion. But in reality they are not under exactly the same influences, for each embryo has its own amnion, and is, therefore, surrounded by a fluid apparently as distinct as though each were in a separate chorion. This forces us to the conviction that the four embryos of a set are derived from a single egg.

If this is the case, one can only conjecture as to the manner in which the conditions seen in the older stages have arisen, and any suggestions which we may have to offer at this time must be taken as tentative. It may be supposed, for example, that the developing egg, after it has reached a stage corresponding to that of an inner-cell mass and trophoblast in other mammals, has, in the case of *Dasypus*, four inner-cell masses — a cell mass for each quadrant; and furthermore that the cells of any given inner-cell mass, together with the trophoblastic cells of its quadrant, are the lineal descendants of one of the blastomeres of the four-cell stage. This possible interpretation receives a striking confirmation in the fact that the four embryos can be arranged into two pairs, the individuals of which approach almost complete identity; and these identicals are not only adjacent to each other, but are also attached to placental areas that are closely united (see Figs. 1 and 2 and table). If all four embryos are derived from a single egg, this is exactly what we should expect to find; for surely the two individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere. If the above be granted, it is an easy matter to explain the origin of the conditions found in late stages; and the condition of a

single chorion with four amnia would receive a rational explanation.

We are, of course, aware that other possible interpretations might be given, such as has been offered from time to time to account for human identical twins. Our studies of the ovarian tissues of *Dasypus* have excluded the most plausible of these, viz., that the ovum may have more than one germinal vesicle. Further investigation will have to settle the next most plausible explanation offered, that the polar bodies may become functional. None of the other suggestions found in the literature seems as credible as the view to which we incline, and in support of our contention we have facts derived from an examination of two sets of ovaries from gravid females. In one set there is but a single corpus luteum, and, while in the other there are two corpora lutea, one has practically disappeared, and evidently had been formed during a previous pregnancy.

After all, the development of the fœtal membranes is a matter of secondary interest, as compared with the more fundamental problem of the identity of the embryos of a set, and its corollary that of sex determination. The bearing of this work on the latter problem is obvious, and we hope that a study of the early developmental stages will lend a solution to this problem, and also furnish a satisfactory explanation of the puzzling question of "identical twins"; and thus raise this explanation from the plane of conjecture to the dignity of observed fact.

AUSTIN, TEXAS,
April 30, 1909.

THE GENERAL BIOLOGICAL SIGNIFICANCE OF CHANGES IN THE PERMEABILITY OF THE SURFACE LAYER OR PLASMA-MEMBRANE OF LIVING CELLS.[1]

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The osmotic properties of living cells have been the subject of repeated investigation since the fundamental experiments of Pfeffer and de Vries, and especially since the rise of the theory of osmotic pressure and the extension of the gas laws to dissolved substances by van't Hoff in 1887.[2] One remarkable result, of the highest significance for general physiology, has been perhaps the main outcome of these researches; living cells of the most diverse kinds have been found to possess in common a high degree of physical impermeability to most of the diffusible non-colloid substances normally occurring in the cells and in their surroundings. The surface film or so called plasma-membrane of cells, while readily permeable to water, offers a highly efficient barrier — at least during the resting or unstimulated state — to the entrance or exit of many dissolved substances of relatively low molecular weight, even of those normally present in protoplasm, as sugars, amino-acids, and neutral salts. This impermeability is a property of the normal living cell, unmodified by experimental conditions. A direct proof of this is the characteristic turgor of plant cells, which is due to the osmotic pressure of crystalloid substances dissolved in the cell-sap; it is clear that the osmotically active substances — sugars, organic acids, and other relatively simple compounds — are ordinarily unable to traverse the limiting surfaces of the protoplasm; were they able to do so the maintenance of the high internal pressures known to exist would be impossible. Such impermeability is a peculiarity of the *living*

[1] From the Physiological Laboratory, Department of Zoölogy, University of Pennsylvania.

[2] A full account of the earlier researches will be found in Hamburger's "Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften," Wiesbaden, I. F. Bergmann, 1902–4. See also Höber's "Physikalische Chemie der Zelle und der Gewebe, 2d ed., Leipzig, Engelmann, 1906.

cell and disappears at death, which is always associated with a loss of turgor and a diffusion of dissolved materials (coloring matters, etc.) from the cell. The increase in permeability may be the consequence, or it may be the cause, of the death-process; at all events it is the invariable concomitant of the latter — a fact apparently signifying that the living condition is incompatible with more than a temporary loss of impermeability.

The dependence of certain fundamental life processes (as growth, which is a manifestation of osmotic energy) on the impermeability of the plasma-membrane is more clearly apparent in plants than in animals. The proof that a similar impermeability characterizes animal cells is due mainly to the use of the plasmolytic method, which was first accurately applied to plants by de Vries, and has later been extended to animal cells by Hamburger, Overton, Koeppe and others. The principle of the method is well known; the turgor-pressure of plant cells, due to the osmotic pressure of the cell-contents, may be compensated by the application of an external osmotic pressure, *provided* that the boundary surface of the protoplast is impermeable also to the external solute; over-compensation, with compression of water from the cell and a shrinkage of the protoplast from the cellulose cell-wall, results if the external pressure distinctly exceeds that of the cell-contents; under-compensation leads to absorption of water; while with equality of external and internal osmotic pressures no osmotic change is seen (cases of hypertonic, hypotonic and isotonic solutions respectively). A means is thus afforded of testing the permeability of the plasma-membrane to different substances. If, using a moderately hypertonic solution of a given substance, a loss of water results which remains permanent during the period of immersion in the solution, impermeability relatively to that substance is indicated (the case of sugars, polyhydric alcohols, neutral salts). If the cell loses water only temporarily, afterwards regaining its original proportions, or even swelling still further, *gradual* entrance of the substance into the cell is indicated; such effects usually appear with solutions of urea, glycerine, or glycol, to which most cells are slowly permeable. If no plasmolysis results, even with strongly hypertonic solutions, a free permeability to the dissolved substance must exist; hence

such solutions typically induce swelling of cells; many organic substances, particularly fat-solvents, belong to this group. Another method of testing permeability has been employed by Hedin, which has the advantage of permitting of quantitative application. A known quantity of the substance to be tested is added to a given volume of a somewhat dense suspension of the cells studied (chiefly blood corpuscles). After an interval the mixture is centrifuged and the partition of the added substance between cells and suspension-medium may then be determined cryoscopically. The results gained by these two methods agree closely. The permeability of animal cells has been studied in considerable detail by various modifications of these methods. Overton in particular has compared the respective permeabilities of animal and plant cells and has shown that essentially the same relations hold for both. The conditions are highly constant and characteristic. All the cells examined have proved impermeable to sugars, polyhydric alcohols (erythrite and higher), amino-acids (glycocoll, alanin, leucin, asparagin, taurin), and most neutral salts of alkali and alkali earth metals; difficultly permeable to dihydric alcohols, urea and glycerine; and freely permeable to monohydric alcohols, ethers, esters, aldehydes, normal and substituted hydrocarbons — in general to such substances as exhibit in common the property of dissolving fats or of being dissolved in them. In applying such methods it must be borne in mind that many substances, including particularly the last named group, alter the normal permeability of the plasma-membrane; allowance has therefore to be made for changes in the normal permeability due to the direct action of the substances investigated. Most of the substances of the first and second of the above groups have, however, relatively slight action of this kind; and impermeability or difficult permeability to these substances appears to be a very general if not a universal rule for both plant and animal cells.

Impermeability to sugars, polyhydric alcohols, amino-acids, neutral salts, combined with ready permeability to those various organic substances which have in common fat-dissolving or lipolytic properties, appears thus to be a fundamental characteristic of living cells. The view that the surface layer of protoplasm is fatty

in nature, early expressed by Quincke and others, has thus been confirmed by a study of the permeability, since fat-solvents will readily enter and traverse a layer consisting largely of fatty substances. Overton has shown that the permeability of cells is in general such as would be expected if the plasma-membrane consisted largely of lecithin and cholesterin, the chief members of the so-called lipoid group of substances, which appear to be constant constituents of protoplasm. He found that those dyes which readily enter cells (*intra vitam* dyes) are very generally soluble in lipoid mixtures or in organic solvents containing lipoids — even if insoluble in triolein and other simple glycerides — while the group of non-vital dyes, particularly the sulphonic acid derivatives, are insoluble in such solvents.[1] A relation between lipoid-solubility and ability to enter cells thus exists for dyes, according to Overton. The rule that such solubility implies ready penetration of a dye into a cell and *vice versa* appears, however, not to be without exceptions;[2] the matter is complicated by the readiness with which the normal permeability may be modified by the very presence of the substance whose penetration is in question. There are, however, other facts which indicate that the distinctive vital permeability of cells is intimately related to the presence of these substances. Chief among these are the peculiar relations of lecithin and cholesterin to hæmolysis, a change which seems to depend primarily on alteration of the permeability of the surface layer of the erythrocytes; the action of various hæmolytic substances (saponin, agaricin, solanin, cobra poison, tetanolysin) is furthered or checked in a characteristic manner by the addition of lipoids to the blood serum. Again, the observations of Pascucci[3] in Hofmeister's laboratory on the permeability of artificial membranes impregnated with lipoids, and on the composition of the stroma of blood corpuscles, afford strong support to the view that lipoids play an essential part in the formation of the plasma-membrane. Naturally the latter is not to be regarded as a simple layer of lipoids; it appears rather to be a surface film of highly complex composi-

[1] Overton, "Jahrbücher für wissenschaftliche Botanik," 34, 1900, p. 669.

[2] Cf. the critique of Brailsford Robertson, *Journal of Biological Chemistry*, 1908, vol. 4, p. 1. Robertson nevertheless regards the surface film as partly composed of lipoids, although he ascribes more importance to the surface film of modified protein.

[3] Pascucci, *Hofmeister's Beiträge*, 1905, 6, pp. 543, 552.

tion, probably consisting of a mixture of substances, chiefly protein but largely lipoid, that have collected at the surface in consequence of their effect in lowering surface-tension. Numerous observations have shown that coherent films may thus be formed at the boundary surface between two fluids.[1] The phenomenon is now recognized as a special instance of the operation of a principle first defined by Willard Gibbs: conditions of equilibrium require that substances which lower the surface-tension of a solvent should collect in the surface layers in higher concentration than in the interior. Proteins and lipoids show this behavior. It must be granted, however, that the conditions which render the plasma-membrane of resting cells so peculiarly impermeable to soluble substances — provided these do not alter its chemical composition or colloidal consistency — are imperfectly understood. The facts of electrical stimulation indicate that the characteristic electrically polarized condition or physiological polarization of the plasma-membrane is a factor of fundamental importance.

During the greater part of its existence the cell thus appears almost completely shut off from osmotic or diffusive exchange with its surroundings. It is evident, however, that this impermeability to the food-materials and to the salts of protoplasm cannot exist at all times and under all conditions; such substances do enter the cell, and since such entrance implies penetration of the surface layer, the latter must under certain conditions become permeable. A specifically physiological problem is here encountered; either soluble food substances reach the interior of the cell quite otherwise than by a simple process of diffusion, or the permeability of the plasma-membrane must at certain times undergo functional alterations so as then to admit such substances. There is in fact evidence that the permeability of the plasma-membrane does increase at times, *e. g.*, under the influence of carbon dioxide, or during stimulation; and it is possible that food materials may enter chiefly or exclusively at such times. Nevertheless, the whole question as to the mode of entrance of soluble food materials into cells still presents many difficulties.

[1] Cf. Ramsden, *Zeitschrift für physikalische Chemie*, 1904, vol. 47, p. 336. Metcalf, *ibid.*, 1905, vol. 52, p. 1. Literature and review in this paper.

The process probably requires the performance of work on the part of cells, just as does the separation of a secretion by a gland. The kidney performs osmotic work in concentrating its secretion, and the cells of the intestinal mucosa absorb many substances against concentration gradients, a capability also implying performance of work. The rapidity with which salts and food-materials are absorbed seems indeed incompatible with the existence of an impermeable plasma-membrane; yet whenever resting cells are tested with regard to their physical permeability the above unequivocal results appear. Evidently the decisive factors in absorption as also in secretion are largely independent of physical diffusion.[1]

Whatever the actual case may be — and the problem remains unsolved for the present — there is no doubt that impermeability to the diffusion of many dissolved substances of low molecular weight is a highly distinctive and even necessary characteristic of living cells. The following considerations will make this clear. Substances of relatively low molecular weight and high diffusibility form an important part of the living protoplasmic complex. In the specific metabolism of any animal the protein and carbohydrate food materials are split respectively to amino-acids and sugars, both highly diffusible substances; and many other diffusible products important in metabolism are formed by oxidation or hydrolysis. These substances must not be lost from the cell. It is plain that any specific organism must exhibit a constant and specific metabolism — is indeed the product or the manifestation of this. Now the existence of specific metabolic processes in any cell requires the presence of many interacting substances in proportions that must not vary widely from a constant mean; in other words, constancy in the character of its metabolic processes is essential to the specificity of a particular cell. Its protoplasm may be regarded as a mixture of diverse yet constantly present substances in an approximate chemical equilibrium of a highly complex order. Any such constancy of composition, implying constancy in the conditions of equilibrium, would be impossible in a system not very completely isolated from its surroundings. In

[1] Cf. Asher, *Biochemische Zeitschrift*, 1908, XIV., p. 1, "Untersuchungen über die physiologische Permeabilität der Zellen."

the cell this isolation is due to the presence of an impermeable surface film. A marked degree of impermeability to the great majority of its diffusible constituents is thus indispensable to the continued existence of a highly complex heterogeneous system like the cell. To certain substances like oxygen and carbon dioxide the plasma-membrane appears quite freely permeable, though even in the case of these substances the permeability seems to differ greatly at different times.[1] In general the conclusion seems justified that the presence of this impermeable boundary is an indispensable condition of the most fundamental chemical characteristics of living matter. We may say indeed that just as the high development of chemistry would have been impossible without the use of vessels in which the interacting substances could be confined and isolated from the surroundings, so the development in phylogenesis of the complex chemical specificity of living organisms has depended on the isolation of the protoplasmic chemical system from its surroundings by a physically impermeable boundary. The impermeability of the plasma membrane thus appears not as a merely incidental character of living cells, but as a primary condition both of their development and of their continued existence.

The non-permeability of cells to many electrolytes is an especially significant characteristic. Neutral salts of the alkali and alkali earth metals are almost invariable constituents of protoplasm; yet the plasma-membrane, as the investigations of Overton and others have shown, is impermeable or difficultly permeable to many if not all of these. This implies impermeability to the undissociated molecules and to one or other or both of the two classes of ions resulting from dissociation. If a membrane is partially permeable to both ions of an electrolyte the chances are that it will be unequally permeable to these; and the case is conceivable that it should be freely permeable to one ion but not to the other. Ostwald[2] in 1890 first directed attention to this possibility; such a membrane, if interposed between two unequally concentrated solutions of the electrolyte, would then be the seat of an electrical potential difference, since there would be a sepa-

[1] The permeability to fat-solvents is an incidental, not a vital, peculiarity, since these substances are not normally present in protoplasm or its surroundings.

[2] *Zeitschrift für physikalische Chemie*, 6, 1890, p. 71.

ration of electricities due to the passage of a certain small proportion of ions of one sign through the membrane; this would continue until the electrostatic tension thus arising balanced the osmotic pressure difference between the ions on opposite sides of the membrane, when the ionic transfer would cease. A certain potential difference — constant (at a given temperature) with unchanged permeability of the membrane and concentrations of the electrolyte — would then exist between opposite surfaces of the membrane. The presence of membranes having such a differential permeability in reference to the anions and cations of the tissues might, Ostwald pointed out, be the source of the electrical phenomena of living organisms; and this suggestion has been further developed by Bernstein, followed by Brünings, Hoeber and others. The theory based on this interpretation — the so-called "membrane theory" of the origin of the bio-electric currents — appears to-day as the most adequate explanation of these complex and puzzling phenomena. Its merit consists mainly in the introduction of a third variable — in addition to character and concentration of the ions at the boundary surface[1] — namely, the ionic permeability of the membrane itself. This is subject to more or less sudden alteration either in the direction of increase or decrease; and the sudden and pronounced electrical changes accompanying stimulation and inhibition, as well as the remarkably high potential differences found in some instances (electric fishes), become readily intelligible on this theory.

The outer uninjured surface of a resting cell — *e. g.*, a muscle cell[2] — always proves positive relatively to the interior whenever the latter is exposed by any kind of injury or by chemical or other alteration of the surface. The membrane theory therefore assumes that the plasma-membrane is freely permeable during rest to the cations of a certain electrolyte or electrolytes contained within the cell, but not to its anions. This condition may, however, undergo temporary alteration, as during stimulation, when there is invariably a fall in the potential difference between exte-

[1] Assuming constant temperature.

[2] Of course observation cannot be directly made on single cells — unless on certain egg-cells — a possibility exemplified by Miss Hyde's investigations cited below. The above statement assumes that what is observed for parallel bundles of cells, *e. g.*, such a muscle as the frog's sartorius, is true for single components of the bundle.

rior and interior of the cell, such as would result from an increased permeability to anions. During life such potential changes occur only temporarily; they are an apparently inseparable accompaniment of any form of stimulation. On the death of the cell, however, there follows a marked and permanent increase in the general permeability, and this change is always associated with a permanent fall in the potential difference between surface and interior. That the normal potential difference observed during life is correlated with the normal impermeability of the plasma-membrane thus appears highly probable. The plasma-membrane is hence to be regarded as the seat, during life, of a permanent electrical polarization which is diminished during stimulation, and disappears or is greatly diminished at death, when the vital semi-permeability is lost. The cation to whose penetration the external positivity is due was at first supposed to be potassium, which is present in relatively large proportion in the interior of vertebrate muscle cells; but this is now known not to be the case. Were it so stimulation would involve a loss of potassium salts from the cell, of which there is no evidence; moreover Hoeber has shown that increasing the external concentration of potassium ions does not reverse the direction of the demarcation current. The indications point strongly to the rapid and highly penetrating hydrogen ion as the source of the outer positive potential of cells; and since acids, particularly carbonic acid, are known to be formed in metabolism and to leave the cell during stimulation, there exists in fact within the cell a constant source of hydrogen ions to account for the characteristic surface polarization. That such a system should show a surface polarization of the above kind is, in fact, not surprising. The depolarization associated with stimulation and with the death process implies the loss of the polarizing electrolyte from the cell; it has long been known that carbonic and possibly other acids are evolved at such times in greatly increased quantity.

Proofs that the surface layer of cells is unequally permeable to the anions and cations of many electrolytes have been furnished by the investigations of Hamburger, Koeppe, and Höber.[1]

[1] An account of these investigations is given in Höber's "Physikalische Chemie der Zelle," pp. 303 *seq.*

These have yielded the result that under the influence of carbonic or other weak acids (*i. e.*, of H-ions in low concentration) blood corpuscles give evidence — by showing an exchange of anions with the surroundings or by characteristic behavior in electrical convection — of becoming much more freely permeable to various anions (Cl, Br, SO_4, HCO_3, NO_3) than before, while the permeability to cations appears unchanged. This increase in permeability to anions is readily reversible on removing the carbon dioxide. Proof is thus afforded that the permeability of cells to ions is a variable quantity, which may undergo increase or decrease according to circumstances. The theoretical importance of a demonstration of reversible changes in ionic permeability is evident when we consider the essential rôle which, on the membrane theory, such changes play in stimulation.

The permeability of the living cell is thus not a constant factor, but one subject to alteration under a variety of conditions. The reversible changes induced by stimulating or inhibiting agencies, whose respective action appears to consist mainly in increasing or decreasing the normal permeability, are of particular physiological interest. Alterations of permeability are also induced by a large class of foreign substances which act directly upon the plasma-membrane. Such are in general: (1) The class of lipolytic substances or organic fat-solvents; these directly affect the lipoids of the plasma-membrane; if present in sufficient concentration they produce marked increase in the permeability of the surface layer of cells. The effect is conspicuous in pigment-containing cells (erythrocytes, egg-cells, etc.); the pigment leaves the cells and colors the solution (hæmolytic action of fat-solvents). Increase of permeability sufficient to allow the exit of colloid substances is usually irreversible and destructive to the cell. (2) Various electrolytes; these affect primarily the aggregation-state of the colloids. Neutral salts of alkali and alkali earth metals produce largely *reversible* changes in permeability; with salts of heavy metals, and with acids and alkalis above low concentrations the effects are irreversible, hence (in part at least) the greater toxicity of this class of electrolytes; alkalis may have also a saponifying action. (3) A large and miscellaneous class of poisonous substances — cytotoxins, hæmolysins, alkaloids and gluco-

sides of various kinds (saponin, solanin, agaricin, quillain, etc.); these increase the permeability of blood corpuscles, liberating hæmoglobin and eventually destroying the cell. Such substances alter the plasma-membrane partly by their action on the lipoids (lipoid-soluble alkaloids), partly by some specific action on the proteins, as apparently in the case of foreign blood sera. (4) Photodynamic substances (eosin, fluorescein, chlorophyll,[1] etc.); these also appear to act in the light primarily on the plasma-membrane, increasing its permeability. Tappeiner's observations[2] indicate clearly that eosin solutions first effect the peripheral layers of the cell. Paramæcia exposed to solutions of the dye in the dark, washed free, and then exposed to the light are but little affected; blood corpuscles show a certain difference in behavior and allow some entrance of the dye in the dark, but hæmolysis is much more rapid if the corpuscles are illuminated while in the solution, than if, after equally prolonged treatment in the dark, they are transferred to an eosin-free medium and then illuminated: *i. e.*, the effect is largely if not exclusively dependent on a surface action. Harzbecker and Jodlbauer[3] find the same general result. Microscopical observation also indicates that the initial stage of the destructive action consists in a disturbance of the osmotic equilibrium between corpuscle and medium, such as would result if the semi-permeability of the plasma-membrane were abolished. The corpuscles first swell, indicating entrance of water; this occurs before any perceptible entrance of the dye. P. v. Baumgarten[4] finds the same initial change in the action of hæmolytic substances, and has referred hæmolysis primarily to a loss of osmotic equilibrium consequent on alteration of the surface layer. This view is also upheld by Hamburger.[5] The increase in permeability, in addition to destroying the osmotic equilibrium, will naturally further the entrance of the toxic substance and the action will then affect the entire cell. In general toxic action must be regarded as depending in many cases primarily on an alteration, particularly an *increase*, in the normal permeability of the cells.

[1] Hausmann, *Biochemische Zeitschrift*, 1909, XVI., p. 294.
[2] Tappeiner, *Biochemische Zeitschrift*, 1908, XII., p. 290; *ibid.*, XIII., p. 1.
[3] Harzbecker u. Jodlbauer, *Biochemische Zeitschrift*, 1908, XII., p. 306.
[4] P. v. Baumgarten, *ibid.*, 1908, XI., p. 21.
[5] Hamburger, *loc. cit.*, vol. 3, p. 360.

Hence the hæmolytic action of many toxins: the injury to the plasma-membranes is naturally not confined to the blood corpuscles — the effect is merely most plainly evident in these cells; but all or most of the cells of the organism are to be regarded as similarly affected.

The above substances produce irreversible or toxic changes in cells by influencing through their chemical or solvent or coagulative action the permeability of the plasma-membrane. Essentially similar effects may result in many cases from moderate rise of temperature. Thus exposure of frog's muscle to temperatures approaching 40° for a short time produces the typical phenomenon known as heat-rigor, in which the muscle shortens and thickens while its substance undergoes complete coagulation and acquires a pronounced acid reaction. A closely similar change results when a muscle is immersed in a saturated solution of a lipoid-solvent like chloroform in an indifferent medium (*e. g.*, Ringer's solution). In both cases the changes in the muscle are associated with a loss of semi-permeability and of electrical polarization; and they are almost undoubtedly direct consequences of this since neither the moderate rise of temperature nor the presence of chloroform has by itself any such coagulative effect on the colloids composing the muscle substance; while loss of semi-permeability, with consequent disturbance of chemical equilibrium and production of acids, may readily produce just such effects.[1] Why such moderate heat should so profoundly alter the properties of the plasma-membrane is not evident at first sight; possibly the colloidal aggregation-state is the condition immediately influenced by the change of temperature; possibly the primary effect is a chemical one.

The above are instances of artificially produced or pathological changes in permeability. Normal or functional changes in this property appear to be of frequent occurrence in living organisms. Thus there is highly conclusive evidence that the general process of stimulation is dependent on a temporary and reversible increase in permeability. Very clear indications of this kind are

[1] Compare my former paper, *American Journal of Physiology*, 1908, XXII., pp. 81–83. Vernon, *Journal of Physiology*, 1899, XXIV., p. 239, and Meigs, *Amer. Journ. Physiol.*, 1909, XXIV., p. 178, also regard heat-rigor as having no direct connection with the heat coagulation of the proteins of muscle.

seen in motile plant organs. The sudden movements of Mimosa leaves, for example, result from a loss of turgor of the pulvinus cells, due to escape of cell-sap — a process which, as Sachs[1] long ago pointed out, signifies temporary increase in permeability. Since the movement is induced by the same stimuli as cause contractions in muscle-cells, and since it is accompanied by a similar electrical variation or action-current (as Burdon-Sanderson first showed), the inference seems natural that the primary change in the stimulation of animal cells is also a temporary increase in permeability. It remains therefore to inquire how such a change could lead to such seemingly disproportionate liberation of chemical and mechanical energy. I have recently discussed the relation of permeability-changes to stimulation in some detail and need not repeat *in extenso* the facts and arguments presented in these papers.[2] Briefly these are as follows: (1) Artificially-induced increase in permeability through the action of various electrolytes, lipoid-solvents, or protoplasmic poisons, produces contraction in muscle cells; (2) the death change, which is associated with an increase in permeability, produces the same effect; (3) the electrical change always associated with stimulation is of a kind indicating temporary depolarization of the plasma-membrane; (4) the loss of irritability at the height of stimulation (refractory period) is what should be expected if the plasma-membrane becomes freely permeable to ions at such times; (5) there are certain inorganic phenomena, showing remarkably close analogies to stimulation, where the effect depends on dissolution of a surface-film (pulsatile mercury and hydrogen peroxide catalysis of Bredig and his pupils); finally (6) the chemical effects of stimulation — increased production of carbon dioxide with increased acidity of the muscle substance, etc.— receive a consistent explanation on physico-chemical grounds if it is assumed that with the increase in permeability the resistance to the escape of the products of oxidation — particularly carbon dioxide — and hence also to the progress of the oxidative energy-yielding reaction is suddenly diminished; the velocity of this reaction hence under-

[1] Cf. Sachs' "Lectures on the Physiology of Plants," 1882. English translation by Marshall Ward, Oxford, 1887, p. 653.

[2] *American Journal of Physiology*, 1908, XXI., p. 200; XXII., p. 75; 1909, XXIV., p. 14.

goes a corresponding sudden increase. Inhibition is to be regarded as the inverse of stimulation and as dependent on still further decrease of the normal resting permeability.

Other instances of functional increase in permeability are apparently seen in gland cells during periods of activity. Certain influences that stimulate or heighten the irritability of muscle cells, as the action of pure solutions of many sodium salts, also, according to Fischer's and J. B. MacCallum's researches in Jacques Loeb's laboratory,[1] increase the permeability of the kidney tubules and of the intestinal epithelium; the effect is checked or counteracted by the presence of calcium salts, as also in the analogous case of muscular twitchings; and according to MacCallum hæmolytic substances — substances that increase the permeability of blood corpuscles — very generally exhibit a diuretic action, *i. e.*, increase the permeability of the kidney cells. The fact that stimulation of a gland through its nerve, as well as of a muscle, has as one of its consequences a characteristic electrical variation also indicates an increase in ionic permeability during stimulation. On the other hand, the recent results of Asher[2] with the salivary gland and the liver do not altogether bear out MacCallum's interpretation, but indicate that with such cells the specific selective power or "physiological permeability" is largely independent of externally induced changes in physical permeability. The selective action of gland cells appears indeed to be their distinctive property, and this must depend on work performed by the gland cell. Nevertheless alterations in physical permeability in all probability play an important rôle in glandular activity, as the conditions in the kidney indicate, although in the case of glands with highly developed selective properties, as the mammary gland or the liver, this factor may appear of subordinate importance.

In a rhythmically automatic tissue like cardiac muscle the phenomena of the action-current indicate on the above theory a regular alternation of periods of increased and decreased permeability corresponding respectively to the contraction and the relaxation phases of the beat. Similar rhythmical changes in permea-

[1] University of California Publications, Physiology, 1904-5, Vols. I., II.

[2] Asher, *loc. cit.*

bility must be assumed to exist in other tissues that show a markedly periodic activity, *e. g.*, automatic nerve centers like the respiratory center, or contractile structures like cilia and contractile vacuoles. The case of cell-division, as seen for example in the cleavage of an egg-cell, is another instance of a rhythmical process which, reasoning from the above considerations, one might expect to find associated with, and perhaps conditioned by, a periodically recurring increase of permeability. It is in fact noteworthy that conditions which produce increased permeability in other cells — as shown by their stimulating or hæmolytic action — very generally initiate cell-division in unfertilized eggs; such are the action of hypertonic solutions, of specific chemical substances (weak acids, potassium salts, alkalis, various coagulative, cytolytic and lipolytic substances), of mechanical treatment, of temperature changes, and of the electric current under certain conditions.[1] Loeb, in a remarkable series of experiments during the past two years, has shown that, in addition to the class of lipoid solvents, such substances as saponin, solanin, bile-salts, soaps, foreign blood sera — in general substances which, as shown by their hæmolytic action, increase the permeability of the surface layers of cells — may initiate cell-division in unfertilized ova. The surface-layer of the egg first undergoes alteration with the separation of a thin film — probably a haptogen membrane consisting mainly of protein material — the fertilization-membrane. Any condition that produces this characteristic change may lead to cell-division. The process by which the membrane is formed is regarded by Loeb as of the same nature as the cytolytic process which follows more prolonged action of the membrane-forming solutions.[2] Cytolysis, however, may be simply a consequence of loss of osmotic equilibrium, as above seen. The significance of the fertilization-membrane has been the subject of much discussion. The mere separation of a thin film from the egg-surface is probably an accessory or accidental feature of the essential change involved; it seems clear, however, that one important effect must result from the removal of such a layer of material from the

[1] Cf. the recent experiments of Delage, *Archives de zoologie expérimentale et générale*, Sér. 4, T. 9, 1908, notes et revue, p. xxx.

[2] Cf. J. Loeb, *Biochemische Zeitschrift*, 15, 1909, p. 269.

plasma-membrane; the latter is, temporarily at least, *thinned* and its permeability will therefore be increased. This increase in permeability is, on the present view, the primary event in the initiation of cell-division.

Why should such increase in permeability — granting that this is the primary change induced by the above various forms of treatment — initiate so apparently complex a process as mitotic cell-division? The main factors in producing this effect are in my opinion two: first, a disturbance of chemical equilibrium due to an increase in the rate at which certain metabolic products (probably chiefly carbon dioxide) are lost from the cell; this is an effect similar to that which, on the general theory of stimulation outlined above, underlies the chemical effect of stimulation in muscle; the precise effect of such a change will of course vary from cell to cell. Second, a definitely localized increase in the general surface-tension of the cell in consequence of a loss or lowering of the electrical surface polarization.

If the entire cell be regarded as a drop of fluid with a distinct tension at its boundary surface, it is apparent that a relative increase in surface-tension over the general surface of the two hemispheres combined with a relative decrease at the equator would result in a drawing of the material toward the two former areas and away from the equatorial region: a division of the drop into two might thus result. Robertson has recently drawn attention to this possibility, and by a simple but ingenious experiment has shown that a floating oil droplet may be made to divide symmetrically into two by locally lowering the surface-tension along its equator by means of a thread containing alkali or soap solution.[1]

[1] Robertson, *Archiv für Entwicklungsmechanik*, 27, 1909, p. 29.

It would seem that the effect of locally increasing the surface-tension would vary with the mobility or fluidity of the substance composing the system. If the surface layer possessed a high degree of viscosity approaching solidity an increase in surface-tension over an equatorial band-like area encircling the cell might produce constriction — just as would occur, *e. g.*, in an inflated rubber ball if the tension were sufficiently increased about its equator. This was Bütschli's supposition, which was later favored by Erlanger and Conklin; and by myself in an early paper. Dividing cells, however, act very plainly like fluid systems (at least in many cases, *e. g.*, starfish eggs), and there are many theoretical grounds for preferring the above point of view (see below). If the egg behaves like a drop of fluid the distribution of surface-tension assumed above would produce the observed change of form.

It can be shown by an application of the Lippmann-Helmholtz theory, connecting variations of surface-tension with changes in the potential difference at the boundary surface between two phases,[1] that increase in the ionic permeability of the plasma-membrane must result in an increase in the surface-tension of the cell. The original theory of Helmholtz[2] — that the tension between (*e. g.*) mercury and the adjoining solution is maximal with zero potential difference, and diminishes as the potential difference between the phases increases — in consequence of the static charge effects at the boundary — has required modification of recent years, since the specific effect of the ions at the boundary appears, apart from the potential difference, to modify the surface-tension.[3] Gouy's[4] extensive studies have shown that various substances, including many non-electrolytes, may modify the surface-tension of mercury. The observed surface-tension in any special case is thus dependent on (1) the specific nature of the adjoining surfaces; (2) the presence of foreign substances at the boundary, and (3) the potential difference across the boundary. In view of the sharply defined discontinuity between (*e. g.*) an egg-cell and its medium the two may be regarded as separate phases,[5] and the tension of the cell surface may be justifiably regarded as subject to the same laws as that of the mercury surface. The nature of the substances at the boundary surface and the potential difference across this surface are thus the factors to be considered. Although both are imperfectly known, certain facts seem well established. The surface film of cells contains fatty substances and proteins, both of which have marked effects in lowering the surface-tension of water; they will thus, in accordance with Gibbs' principle, tend to collect at the boundary, and the characteristics of the plasma-membrane are no doubt largely due to this

[1] Lippmann, "Relations entre les phénomènes electriques et capillaires," *Annales de chimie et de physique*, 5me Série, T. 5, 1875, p. 494.

[2] Helmholtz, "Gesammelte Abhandlungen," Bd. I., 1882, p. 925.

[3] Cf. S. W. J. Smith, *Philosophical Transactions*, Series A, 1900, 193, p. 47. Also the papers of Warburg, G. Meyer, and Paschen.

[4] Gouy, *Annales de chimie et de physique* (7), vol. 29, p. 145; (8), vol. 8, p. 291, and vol. 9, p. 75.

[5] The criterion for regarding two contiguous systems as distinct phases is essentially that they shall be bounded by a definite surface of discontinuity exhibiting characteristic energy relations (surface tension, etc.).

tendency. The presence of such substances implies that the surface-tension of cells is at least much lower than that of pure water in contact with air; it will be still further lowered in consequence of the electrical surface polarization. The surface-tension of protoplasm is thus undoubtedly very low, and apparently under circumstances may become even negative (*e. g.*, formation of pseudopodia); the low value of this tension is shown by the readiness with which minute portions of protoplasm — amœbæ, egg-cells, leucocytes, etc. — despite the large ratio of surface to volume, undergo flowing and spreading movements. Electrical stimulation produces rounding of amœboid cells — a result to be expected if the stimulus has a depolarizing effect with consequent increase of tension; cold produces a similar change of form — a consequence, possibly, of the characteristic negative temperature-coefficient of surface-tension.

A part of the low surface-tension of cells is to be ascribed to the existence of an electrical surface polarization. The evidence that this condition characterizes all living cells, while largely indirect in the nature of the case, must on the whole be regarded as strong; wherever a directly electrical test is practicable a potential difference between exterior and interior of living cells may be shown to exist, with outer surface positive; on stimulation, death or injury this potential difference undergoes a decrease.

The explanation why increase of ionic permeability produces partial or complete depolarization or fall in the potential difference between exterior and interior of the cell is briefly as follows. It is assumed that the plasma-membrane represents a partition freely permeable to the cations (probably hydrogen ions) of certain intracellular electrolytes (chiefly carbonic acid), but impermeable or difficultly permeable to the anions (assumed to be mainly HCO_3^- and $CO_3^=$) and to the undissociated molecules. This means that the velocity of the cation is practically unmodified by the membrane while that of the anion is greatly reduced. Under these conditions there will be a marked difference in the concentrations of the electrolyte on opposite sides of the membrane; the potential difference between the two solutions will be that observed between unequally concentrated adjoining solutions of an electrolyte with ions of unequal velocities. This potential difference E

according to Nernst's formula[1] may be estimated from the following values:

$$E = \frac{u - v}{u + v} \frac{RT}{q} ln \frac{c_2}{c_1}.$$

Generally, therefore, the greater the difference between the two ionic velocities the greater the potential difference across the boundary; hence this will be maximal with complete impermeability to the anion, since then its velocity is reduced to zero at the membrane and $u - v/u + v$ becomes unity. Normally, however, there is probably a certain permeability to the anion, since carbonic acid is continually evolved from living cells, although slowly in a state of rest; the potential difference in resting cells may thus be supposed to approach but not to reach the maximum. If then the permeability undergoes an increase so that anions may penetrate the membrane with considerable freeness, *i. e.*, with increased velocity, the value of $u - v/u + v$ and also of c_2/c_1 will decrease; the result will be a fall of the potential difference across the surface. The extent of this fall will obviously depend on the degree of the increase in permeability and may show a considerable range of variability.

With decrease in the potential difference the surface-tension must undergo a corresponding increase; this increase in surface-tension is, on the present theory, the main condition of the form change in cell-division. Other changes of form may naturally be similarly conditioned; irregular form changes are frequent in unfertilized eggs in which division is induced by the artificial parthogenetic methods; regular cleavage would require a symmetrical distribution of the areas of increased permeability with reference to the future cleavage plane.

In agreement with the above view of the nature of the chemical effects of stimulation, the initiation of the characteristic series of chemical processes in cell-division is to be referred to a disturbance of chemical equilibrium in consequence of the more rapid loss of certain materials, perhaps simply carbon dioxide,

[1] E is potential difference in volts, u velocity of cation, v that of anion, R gas constant, T absolute temperature, q quantity of electricity (coulombs) carried by a monovalent gram-ion, *ln* natural logarithm, c_1 and c_2 concentrations of the dissociated part of the electrolyte on opposite sides of the boundary surface. The ions are assumed to be monovalent in this form of the formula.

through the now permeable plasma-membrane. According to Lyon's[1] observations the carbon dioxide production by the dividing egg exhibits a rhythmical increase and decrease running parallel with the rhythm of cell-division; the periods of increased carbon dioxide production probably correspond to periods of increased permeability.

Such changes ought, on the membrane theory, to be accompanied by changes in the state of electrical surface polarization. Experimental proof of the existence of such changes is difficult but probably not altogether impracticable. Miss Hyde has attempted — using the capillary electrometer, with one electrode on the blastodisc of dividing fundulus eggs, the other on the opposite pole, — to determine if, during cell division, any potential difference makes its appearance between the dividing portion of the egg and its unaltered general surface.[2] While her results are not altogether unequivocal, they do seem to indicate that at or about the time of cleavage the outer surface of the blastodisc becomes negative relatively to the general outer surface of the egg — *i. e.*, undergoes a change of potential similar to that which occurs during stimulation, indicating increased permeability. These important and interesting experiments should be repeated and confirmed. There are in fact various indications that during cell-division the potential difference between the exterior and interior of the cell undergoes marked alteration. The disposition of the colloidal material of the cell at this time in the characteristic radiations is strong confirmatory evidence; this phenomenon plainly suggests the polarization of colloidal particles in a strong electrical field. It is a corollary of the above theory that with the appearance of an increased permeability — implying depolarization of the surface layer — the peripheral regions of the protoplasm must become — for a time at least, until the potentials are equalized — *positive* relatively to the interior. If the voltage of the surface potential change is comparable to that of the action-current of muscle, *i. e.*, *ca.* 0.05–0.08 volt (as is highly probable), a potential gradient of considerable steepness will exist temporarily, at the time of increased permeability, between the

[1] E. P. Lyon, *American Journal of Physiology*, 1904, XI., p. 52.
[2] I. H. Hyde, *ibid.*, 1904, XII., p. 241.

peripheral and the central regions of the protoplasm; this may be amply sufficient to account for the striking change in the configuration of the cytoplasmic colloids.[1] The peripheral layer would become positive relatively to the central region under the conditions existing in such a system as a cell with a surface polarization of the kind indicated. If we can draw safe inferences from the cytological facts the astral centers do in fact show the properties of negative regions.[2] I hope however to consider the physiology of cell-division from the above point of view more fully in a later paper.[3]

[1] It may be desirable to give here the estimate on which this statement is based. If we assume that the surface potential difference due to the physiological polarization has the same value as the potential difference of the demarcation current of muscle (*ca.* 0.08 volt) and that the fall of potential or negative variation during cleavage is equal to that of the action current (*ca.* 0.05 volt), we infer that in the resting cell there is a potential difference between exterior and interior equal to *ca.* 0.08 volt. This gradient, being due mainly to the unequal permeability of the plasma-membrane to the two ions, exists almost entirely between the inner and outer surfaces of the plasma-membrane; the interior of the cell (cytoplasm) would thus have an almost uniform negative potential of about this value with little if any fall between its central and peripheral regions. If now, in consequence of a sudden increase in permeability, the potential difference across the plasma-membrane decreases by 0.05 volt, a gradient will temporarily exist between peripheral and central regions of the protoplasm equal to this value, the most negative region being that most remote from the periphery. A gradient of 0.05 volt between the center and the periphery of a sea-urchin egg, diameter 0.072 mm., is a gradient of 0.05 volt in 0.036 mm., *i. e.*, 1 volt in 0.072 cm. or about 14 volts per centimeter. The assumed potential differences may be too high, though they are typical of the directly observed values in muscle cells. The change in permeability is also probably rather gradual than sudden. At least these considerations show that a strong electrical field may arise between central and peripheral regions of the cell under the conditions assumed. Of course no such potential difference could be *maintained* without the performance of electrical work on the part of the cell (assuming the free movements of ions within the cytoplasm); but its existence for only a few seconds might well produce the observed effects. This note, however, aims merely at justifying the above statement in the text, not at a complete discussion.

[2] Cf. my paper in *American Journal of Physiology*, 1905, XV., p. 46.

[3] In my first paper dealing with the theory of cell-division (BIOLOGICAL BULLETIN, 4, 1903, p. 175) the system of astral radiations was recognized as evidence of an electrical potential difference between peripheral and central regions of the dividing cell, and the form change was ascribed to a capillary electric change of surface-tension. The tentative hypothesis then presented to account for the general course of the process was, however, quite different from that outlined above, and, with the exception of certain details, will have to be abandoned.

A REËXAMINATION OF THE CYTOLOGY OF HYDRACTINIA AND PENNARIA.[1]

W. M. SMALLWOOD.

The present report does not claim to be a complete discussion of the cytological phenomena in the developmental history of *Hydractinia* and *Pennaria*, nevertheless, it adds considerable new data hitherto unpublished concerning the problem of the structure of the egg, the migration of the chromatin, maturation, fertilization and mitosis. However, the present paper cannot claim to settle the controversy as to the existence of amitosis because the results concerning which there may be some differences of opinion have been negatively interpreted.

During the summer of 1906 Professor C. W. Hargitt asked me to undertake a reëxamination of some of his work on hydroids paying particular attention to the cytological phenomena of the early development, that being the point of most interest. The results of Bunting, '94, on *Hydractinia* were not in agreement with his observations on *Pennaria*, *Eudendrium*, *Clava*, etc., so that he suggested that it might be well to restudy this form also. While I have had opportunity to examine all of Professor Hargitt's preparations on the several hydroids studied by him, it seems wise to confine this paper to *Hydractinia* and *Pennaria*.

It is not often that such a study as this is undertaken and when it is there is involved a great deal of work that it would be superfluous to republish so that the present paper needs to be read in connection with Bunting, '94, on *Hydractinia* and Hargitt, '04, on *Pennaria*. At the beginning of this study, Professor Hargitt explicitly stated that he wished to give me absolute freedom in the problem and the interpretation of the results. This he has done even to the extent of not seeing any of my preparations until I turned over the finished paper to him.

When he asked me to undertake this restudy, he volunteered to collect and preserve the material necessary. In this particular

[1] Contributions from the Zoölogical Laboratory, Syracuse University, C. W. Hargitt, director.

too much credit cannot be given Professor Hargitt for his persistent experiments in trying to find a suitable fixing reagent for these refractory eggs of hydroids. Had I been without the benefit of his long experience, I doubt if the present results could have been secured. He used, among fixing agents, Bouin's fluid which has given the best fixation of any thus far tried and certainly these preparations are superior in regard to their fixation to any which he made during the preceding years of study. There is very little doubt that the eggs of hydroids degenerate when left in alcohol for some time and should be embedded in paraffin as soon as possible.

It seems unnecessary to review the general literature on this subject for this has already been well done by Hargitt in his numerous papers and by Bigelow, '08. Professor Hargitt is so well known as an authority on hydroids that it has seemed unnecessary for me to make an elaborate critique of his papers. Therefore, I have in most instances simply referred to the pages where he discussed similar phenomena, elaborating only those points upon which I have additional data.

Origin of the Eggs in *Hydractinia*.

The eggs arise in the entoderm close to the basement membrane. Certain entoderm cells are directly transformed without any immediately preceding cell division. These young ova are distinguished by having a large nucleus and granular cytoplasm. No special region in the polyp is devoted to the production of ova as Bunting, '94, maintains and text-figures 1 and 2 demonstrate. The young ova are as likely to begin their growth in the cells in the base of the polyp as in those along the side.

Growth of the Egg in *Hydractinia*.

The first apparent change in these entoderm cells which are to become ova that has thus far been observed is a marked increase in the size of the nucleus before the cell as a whole has undergone any change. The result is that the nucleus occupies most of the cell, the cytoplasm being limited to a narrow border. A comparison of the nuclear contents at this early stage with the surrounding entoderm cells shows that the nucleolus is increas-

ing rapidly in size. In the entoderm cells it is so very small that it is difficult to be sure of its presence in some cells, and the same is true in some of the young ova, but in most instances it is a clearly defined and easily distinguished body.

The most interesting change is found in the chromatin network. In the young ova it is now in its most conspicuous state. From

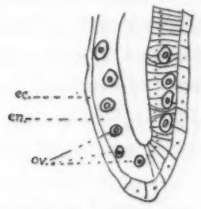

FIG. 1. Outline drawing of base of polyp to show the position of the ova. *ec*, ectoderm; *en*, entoderm; *ov*, ova. *Hydractinia*.

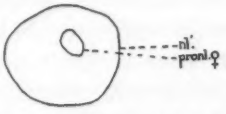

FIG. 3. Camera lucida drawing of the egg nucleus, the larger circle, and the female pronucleus, the smaller circle. *Nl*, nucleus; pronl. ♀, female pronucleus.

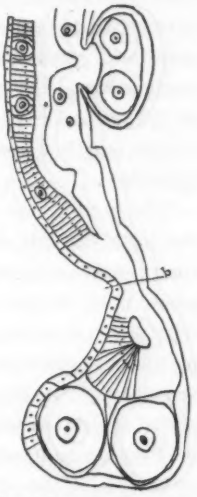

FIG. 2. Outline drawing to show that sometimes the Hydractinian polyp branches. *B*, base of polyp. Reconstructed from several sections.

this time on until new ova arise in a new polyp the chromatin does not possess such distinctness. A loose network is distributed through the nucleus with conspicuous masses where the threads cross. The achromatic substance (residual substance of Lillie, '06) does not stain in the acid or basic stains. The nuclear membrane is very distinct, chiefly due to the fact that a considerable amount of chromatin material seems to be directly in contact with it. The cytoplasm is as yet free from the microsomes — minute granules — which characterize the slightly older ova.

The next step in this gradual transformation is the rapid increase in the cytoplasm, accompanied by a growth in the nucleus. The cytoplasm is now loosely sprinkled with microsomes, in

places giving the appearance of a reticulum. With the accumulation of the microsomes, the reticulate condition is obliterated and the cytoplasm becomes a dense mass of microsomes. In such young ova the achromatic substance of the nucleus takes a plasma stain such as Orange G or Bordeaux red. The vacuolation of the nucleolus has begun by this time, the vacuoles taking the plasma stain. This vacuolation of the nucleolus is in agreement with the many cases already described. The vacuolation continues until the nucleolus disappears which occurs before maturation. The growth, vacuolation and eventual disappearance of the nucleolus is an event which takes place during the growth of the egg, but is not synchronous with any definite phase of this growth.

The young ova thus described are found in the entoderm at the base, the side of the polyp, or in the gonophore. About this time the ova take their permanent position (Bunting) in the gonophore, *i. e.*, in the ectoderm where they increase greatly in size. This increase in size is largely a matter associated with the growth of the individual microsomes into small spherules. We are led to believe from this study that there are also many microsomes added which likewise are changed into spherules. These spherules are so numerous in the adult egg as to conceal the ultimate structure of the cytoplasm. They take a dense stain but not always a homogeneous one. During this gradual growth, the staining reaction changes, a change which can be readily seen on a slide where all stages are represented. A number of such were studied where all of the material had received the same treatment from fixation to and including staining. On such a slide the young ova are so deeply stained as to conceal most of the details of structure while the large eggs show but a faint response to the stain. But when the more mature eggs are once stained in iron hæmatoxylin, it takes considerable time to differentiate them in the iron so tenaciously do they hold the stain. Under these conditions one may see on the same slide young ova black and blue-black in color while the older eggs are hardly stained at all. Between these two extremes, there are all gradations. This change in color reaction is evidently due to the transformation of the microsomes into spherules.

Chromatin Changes in the Egg of *Hydractinia*.

While the cytoplasm is becoming thus transformed, the nucleus has increased in size although it does not become more distinct. The nucleolus is mostly occupied by a large vacuole. In place of the sharply defined chromatin masses in the younger ova, there is a marked change in this particular. The typical chromatin reaction is hardly evident and the whole nucleus tends to take a stain similar to the cytoplasm. The chromatin threads and masses are less definite in position and arrangement. The question naturally arises, what is becoming of the chromatin? Can its disappearance be traced into any part of the egg? In other words, is there a definite and specific migration of chromatin from the nucleus into the cytoplasm? The following facts are submitted in answer to these queries.

While the eggs are still in the gonophore and the nucleolus is becoming vacuolated, small particles of chromatin leave the nucleus and wander out into the cytoplasm. Fig. 1, Pl. I., shows the early stages of this process, some of the chromatin granules are just emerging. Others have moved some distance. My attention was first directed to this phenomenon by finding eggs which showed conditions such as Fig. 2, Pl. I., typifies. A number of densely staining chromatin granules are scattered in the cytoplasm and mostly surrounded by a narrow clear area. These masses of chromatin are small and usually single but occasionally two or three are found in a single vacuole. The reason for regarding these masses as chromatin is because they give the same color reaction as similar shaped bodies in the nucleus; and for the further reason which is obvious in Fig. 1, namely, the actual migration of the chromatin from the nucleus.

When the size of the nucleus of the mature egg, *i. e.*, before maturation, is compared with the female pro-nucleus one is strongly impressed with the great reduction in size. Text-fig. 3 is a camera lucida drawing of the outline of the egg nucleus as represented by the larger circle, while the smaller circle within represents the size of the female pro-nucleus. It must be apparent at once that up to and during maturation there is a remarkable reduction in the size of the nucleus which the maturation phenomenon alone does not adequately explain. The nucleus

loses a large amount of chromatin by direct migration into the cytoplasm which is entirely independent of the chromatin discharged during the formation of the polar bodies. The subsequent fate of this discharged chromatin has been studied with much pains and it is the belief of the writer that it is something as follows: When the cytoplasm of the egg is examined after it has been discharged from the gonophore there appear many areas that are free from the characteristic granules of the surrounding cytoplasm. These areas are usually round and contain small particles that stain with Borax carmine or hæmatoxylin. They look so much like faintly staining nuclei that their appearance is very confusing at first (Fig. 6). As segmentation progresses, these areas tend to migrate to the periphery of the egg and are occasionally so numerous that they form a nearly continuous row around the embryo, Fig. 9. Eventually they are absorbed by the cytoplasm. This explanation, then, traces the highly vacuolated condition of the cytoplasm in *Hydractinia* directly to the migration of chromatin from the nucleus before maturation begins. A similar series of changes occurs in *Pennaria* but at a different time.

Localization of the Formative Stuffs in *Hydractinia*.

The following extract indicates the extent of the previous description of the structure of the cytoplasm. "Sections of the egg show deutoplasm spheres distributed throughout the protoplasm, with the exception of the outer rim which is composed entirely of protoplasm" (Bunting, page 215).

The cytoplasm exhibits a rather definite localization of the so-called formative stuffs in the presence of a coarsely granular crescentic area (picro-acetic fixation) located on the side of the egg in which the nucleus lies—the animal pole. The appearance of these granules in *Hydractinia* is very similar to what Hargitt, '06, p. 214, has found in *Clava*. In addition to these bodies there are some minute bodies located around the periphery of the egg in a narrow band which takes a deep blue stain (Borax carmine, Lyons blue method). These particles do not stain readily and so were overlooked by Bunting. By the regular hæmotoxylin methods they are usually indistinguishable from the other micro-

somes and spherules. This gives three distinct bodies in the cytoplasm: (1) The ordinary bodies all through the cytoplasm and usually interpreted as yolk masses; (2) the coarse granules distributed in crescentic bands; (3) the small bodies around the periphery. The small peripheral granules remain on the outside of the embryo during cleavage and can be traced into the planula. In the planula they are confined to the ectoderm. The differentiation and subsequent fate of similar particles has been made out in *Pennaria*. The first and second class of granules are chiefly confined to the mass of cells within the ectoderm of the planula, although a few scattering granules are seen in the ectoderm.

Maturation in *Hydractinia*.

The following quotation reveals the extent of previous observations on this phase of development. "The ovum while in the gonophore has a well-defined nucleus situated above the center of the egg, which fades from view when the ovum is deposited" (Bunting, page 215). "In about fifteen minutes after the ova are laid the polar bodies appear. When first observed two globules were present, one had been extruded, while the other one was just appearing. One of the two divided subsequently, in a plane at right angles to the first cleavage plane of the ovum. Within ten minutes from the extrusion of the first polar body, the second was ejected" (Bunting, page 216).

The nucleus during growth varies from round to elliptical and in this latter shape the ends may nearly reach to the periphery but until maturation begins, the nucleus is central in position. It is to be regretted that more stages in maturation have not been discovered notwithstanding the fact that large numbers of slides have been made of eggs just after deposition. Maturation begins before the eggs are discharged from the gonophore but just how long I am unable to state. The fact that this process begins while the eggs are still retained, makes the solving of the problem tedious in as much as the gonophores are not set free as are the medusæ in *Pennaria*. Occasionally a gonophore containing eggs is found among the recently discharged eggs and it was in such that the first signs of maturation were detected. A large number of slides were made of the large gonophores from colonies that

were laying eggs when fixed but none of these showed any of the maturation phenomena. Fig. 3, Pl. I., shows the prophase of the first maturation. The asters have moved part way around the nucleus and a few spindle fibers are evident. The nuclear wall on the side toward the middle has been partly broken down. The chromatin shows but a slight tendency to take a stain although it is collecting into definite masses. The chief importance of this drawing centers around the process by means of which maturation occurs, namely, the mitotic process.

When this material was collected it was not thought that maturation began until after deposition because of the observations quoted. For that reason much time was spent studying the eggs just after deposition, but in all cases none of the earlier stages were found. In two or three eggs undoubted polar bodies were found after the eggs had been deposited, and such a case is shown in Fig. 4, Pl. I. Fig. 4 represents the late telophase of what I judge to be the second maturation. In the polar cell there are several vesicles which probably represent chromatin. In the egg the chromatin vessels are very small and grouped among the remains of the breaking down astral fibers. These vesicles collect into a single vesicle, the female pro-nucleus, Fig. 5, Pl. I.

Certainly these two figures do not furnish a complete account of maturation, but they do show: (1) The nature of the process; (2) that this process begins in the gonophore; (3) that Miss Bunting's observations are not correct, because if what she observed were polar bodies they would be found attached to the egg before and after segmentation, but such is not the case. The polar bodies usually drop off as soon as the eggs are discharged. In all of the eggs studied but two were found which retained the egg nucleus after the egg had been deposited.

One of the curious conditions is shown in Fig. 5, where the female pro-nucleus lies next to the periphery of the egg with a few fibers apparently starting from its outer pole. This was thought for some time to mean the prophase in maturation, but never could fibers or an aster be found at the inner pole. Just why there should be a small furrow over this nucleus I do not know, and such a furrow does not always occur. It might be

thought to mean the beginning of segmentation, but such a furrow may appear before fertilization takes place. In a number of instances the nucleus in this same stage was seen to be partly protruding from the substance of the cytoplasm, a condition for which no explanation is offered. The chromatin in the female pro-nucleus takes a faint stain up to the time of the first cleavage. The differentiation of the egg of *Hydractinia* is very difficult, much more so than in *Pennaria*, which makes the recognition of these very arduous. In the region of the nucleus in Fig. 5 there are several deeply staining particles which look much like chromatin, but of their nature I am uncertain. That this nucleus is the female pro-nucleus the following reasons indicate: (1) The absence of the nucleolus; (2) its relatively small size; (3) that it has been traced directly into the first cleavage.

Fertilization in *Hydractinia*.

Thus far no sperms have been found in the eggs before they were deposited but after deposition spermatozoa are seen in contact with the eggs. The sperm head becomes transformed into a vesicle soon after it penetrates the cytoplasm. There does not seem to be any definite place where the sperm enters the egg. During the progress of the male pro-nucleus through the cytoplasm, no aster was seen nor any definite path. The staining reaction of this body is so very faint that it is made out only after careful study with the oil immersion.

Fig. 6, Pl. I., shows the approach of the male pro-nucleus and the change in shape of the female pro-nucleus preparatory to the prophase of the first segmentation. No asters or radiations could be distinguished in connection with either of these pro-nuclei at this stage. These observations further show that the egg nucleus does not "fade from view when the ovum is deposited" but that it can be traced continuously from the egg in the gonophore to the first segmentation stage.

Cleavage in *Hydractinia*.

The difficulties encountered in differentiating these eggs has made it almost impossible to discover a complete series of the changes in any of the stages as many of the mitotic phenomena

are visible only with the oil immersion lens. This means that but few eggs will be cut in just the right plane to enable one to make out the correct relations ; and it also means that important conditions will escape detection.

The first division of the egg into the two-cell stage is preceded by the formation of a definite mitotic figure after the male and female pro-nuclei have come together. The chromatin becomes more responsive to stain and gradually condenses into a definite number of very minute chromosomes. These chromosomes in size and staining reaction are so similar to some of the granules in the cytoplasm, Fig. 8, Pl. II., that it is impossible to be certain that they are chromosomes unless the spindle fibers are present. This makes the determination of the number of chromosomes difficult because when one has a cross-section of the metaphase through the equatorial region, one cannot determine the relation of the spindle fibers to the chromosomes and so cannot be certain of their number. The task was a little easier in *Pennaria*, where fourteen were counted in the anaphase, although here I am not certain that this is the correct number. I think that there are from twelve to sixteen chromosomes present in these hydroids, the exact number remains to be determined.

The chromosomes form in the typical metaphase condition, split and move toward each pole of the spindle. In the anaphase distinct interzonal fibers are present. During the telophase the chromosomes are transformed into a nucleus. This nucleus does not necessarily assume the rounded outline but is often elongated and even irregular in shape. The prophase of the next cleavage frequently begins while the nucleus is in this condition, Fig. 7, Pl. I. In Fig. 7 a typical prophase of mitosis in cleavage is shown. The faint asters are on opposite sides of the elongate nucleus, and a few spindle fibers are forming preparatory to the metaphase and the dissolution of the nucleus. The centrosphere as shown in Figs. 7 and 8 will be discussed in a separate section.

A definite mitotic figure has been made out in all of the early stages of segmentation and followed up to the planula stage. A typical condition of the early embryo is shown in outline in Fig. 9, Pl. II. The cells surround a cavity which is first seen in the two-cell stage and is due to the separation of these first two cells.

This cavity increases in extent as segmentation continues. After a time the peripheral cells begin to segment in such a manner as to set cells free in this cavity. The direction of the spindle in Fig. 8 shows the method. Fig. 8 was taken from one of the cells shown in Fig. 9 and in each of the remaining blastomeres there is a mitotic figure, so placed as to give rise to a cell that becomes free in the segmentation cavity. The segmentation cavity finally becomes full of cells due to the setting free of cells from the periphery and the subsequent division of these same cells as they lie in this cavity.

Membranes in *Pennaria*.

First or False Membrane.—The absence of a membrane or membrane-like structure in the animal egg is doubted by some notwithstanding that Wilson, '82, Metschnikoff, '86, Hargitt, '04, and others have repeatedly stated that the eggs of hydroids are naked. Brauer, '91, finds in *Hydra*, after the embryo is formed, two membranes produced by the ectoderm, but the unsegmented egg is naked. Previous studies in *Pennaria* make no mention of an egg membrane, but the ectosarcal portion of the egg is described by Hargitt as forming at times a filamentous membrane around the cytoplasmic papillæ. Aside from these references no mention is made of membrane in hydroids.

When the eggs of *Pennaria* are well fixed in Bouin, and a hæmatoxylin stain is followed by a plasma stain such as Bordeaux red, a rather broad but uneven structure is readily observed. It first forms while the egg is still growing and is easily made out while the eggs are still in the medusa. The portion of the egg adjacent to the manubrium shows pseudopodia-like processes (cf. Hargitt) and between these processes this structure is quite wide and irregular in width. After the eggs are deposited and have assumed the rounded outline, this membrane-like structure appears as shown in text-fig. 4. It is rarely of uniform thickness and usually shows one place that is bulging. Also

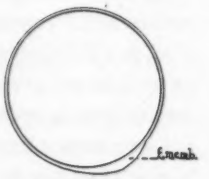

Fig. 4. Outline drawing to show the relative thickness and variability in width of the false membrane. *F. memb*, false membrane.

in the laid eggs as segmentation begins and continues, it appears torn so that some of the eggs will be only partly surrounded by it. Some time during the progress of cleavage this membrane disappears entirely. It is well known that the newly deposited eggs of *Pennaria* are inclined to stick to bits of grass, a glass dish, etc., which is due to the adhesive property of the false membrane. The term false membrane is applied to this structure because it is not permanent; and the term membrane is used because it seems to serve the purpose and do the work of a real membrane. No differential lamellæ were visible in this false membrane either before or after deposition. Numerous sperm heads are frequently to be distinguished within its substance. The inequalities in thickness of the false membrane suggest that this substance is of a fluid nature in the living egg and transparent. If this interpretation prevails, then one can no longer speak of the eggs in *Pennaria* as being naked.

Second or True Membrane. — After fertilization and with the beginning of cleavage a second membrane begins to form (cf. Hargitt) which lies in close contact with the granules of the cytoplasm and completely surrounds each cell as it is produced in cleavage. This structure compares favorably in appearance, staining reaction, etc., with the regularly described egg membranes of animal eggs and so is designated as the true membrane.

Maturation of the Egg in *Pennaria*.

From the early development of the egg up to the beginning of maturation I cannot add any new data, but do confirm Hargitt's ('04, p. 456) observations. The extensive observations of Hargitt as well as my present independent studies indicate that it is very rare to find a polar body attached to the egg after deposition, not one in one hundred will show a polar body at this time. It is also very rare to find a deposited egg that still retains the egg nucleus with a nucleolus. In fact this latter structure, the nucleolus, has been taken as an indication of whether maturation has taken place or not. Where it is lacking I have designated the nucleus as the female pro-nucleus. The egg nucleus in *Pennaria* does not show any such great size as is found in *Hydractinia* and varies but slightly from the female pro-nucleus which is

always present in the egg previous to the beginning of segmentation.

Sections of medusæ after they have become free from the colony and before the eggs are discharged may show the presence of polar bodies. Several different slides show conditions such as are indicated in Fig. 10, Pl. II. The first polar body is being shoved to one side by the formation of the second. The chromatin is in the form of three vesicles in the first polar body. Even while the egg is in the medusa, the polar body may be pushed some distance from the spot where it emerged which may be due to the contractions of the bell of the medusa. The second polar body has ten vesicles in two groups; while thirteen are made out deeper in the egg. To the right there is one isolated vesicle. This one and several of the thirteen contain granules of chromatin. This interpretation of the vesicles grows out of my

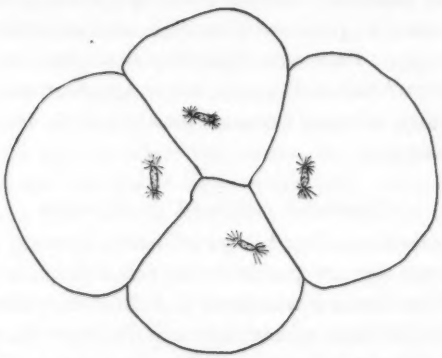

FIG. 5. A regular segmentation stage, all cells in prophase.

study of the changes through which the chromatin passes during cleavage. The several vesicles deep in the egg unite into a single definite nucleus like the one in Fig. 11, Pl. II. That these polar bodies are formed by the mitotic process, there is but little doubt because of similar conditions in *Hydractinia* where there can be no doubt as to the beginning of the process; and because of the state of the chromatin, which in this condition of several vesicles, is entirely unlike the amitotic process of division.

In the same medusa from which Fig. 10 was taken there were

three other eggs in the same phase of maturation. *Pennaria* was collected by Professor Hargitt early in the morning and in the afternoon but in the numerous sections made of the adult medusæ, none showed any earlier stages. With this data at hand, however, it should be an easy matter to secure the intervening stages which undoubtedly occur about the time the medusæ are set free. Sections of medusæ still contained one or two eggs which in each instance had completed maturation, Fig. 11, and the polar bodies were not in contact with the egg. The conditions shown in Figs. 47 and 49 by Hargitt are without question polar bodies and probably the second. The state of the chromatin vesicles in Fig. 47 is more regular than any that I have seen which may be due to the preservation. They seem also to be more scattered than any observed in eggs fixed in Bouin. The clear area within the egg just beneath these vesicles was not seen in the Bouin fixation. Another puzzling feature of this study on maturation was the presence of small bodies attached to the surface of the egg. They were especially noticeable on the eggs of *Hydractinia*. After some study, it was apparent that they were protozoa which in many instances looked exactly like polar cells found in mollusca.

Chromatin Changes in *Pennaria*.

The female pro-nucleus is a small, faintly staining body that is found in eggs that are just laid and many that are still in the medusa. Sometimes it was pointed at the outer end and pushed close against the false membrane but it was never found protruding. It occupies this position until the approach of the male pro-nucleus when it may move some distance from the periphery of the egg. But before the fusion of the two pro-nuclei, there occurs a series of unusual changes in the chromatin, especially of the female pro-nucleus. Changes of a similar nature but not as extensive are found taking place in the male pro-nucleus. The chromatin changes described in *Hydractinia* preceded maturation. These in *Pennaria* follow maturation.

It is difficult to determine whether there is any order to these changes and so no attempt is made to decide which is the older state in the series of Figs. 12 to 18. In several of these figures

the male pro-nucleus is shown but its position, near or far from the female pro-nucleus does not seem to influence the time when the chromatin is to migrate from the nucleus. Immediately in contact with each pro-nucleus, the cytoplasm becomes denser and is composed of finer granules (cf. also Hargitt, Figs. 48, 49, 50). But isolated or wandering nuclear vesicles usually lack this modification of the cytoplasm which has been used to assist in recognizing the pro-nuclei.

Figs. 12, Pl. II., 15 and 18, Pl. III., show some of the chromatin granules close to the nuclear membrane as if they had just emerged from the nucleus into the cytoplasm. In both Figs. 15 and 18 these chromatin granules are on opposite sides of the nucleus which indicates that their position is not the result of the accident of cutting the sections. The male pro-nucleus in Fig. 15 shows the same condition of the chromatin. After the chromatin has been in the cytoplasm for some time, there are found definite small vesicles usually empty. From the study of mitosis in cleavage and the changes in the chromatin during the anaphase and telophase, the suggestion that these vesicles are the product of the transformed chromatin seems inevitable. During this period, while the chromatin is migrating into the cytoplasm, the chromatin both within and without the nucleus takes a very faint stain so that the whole nucleus is easily overlooked. Some of the most satisfactory results were obtained by using Brazilian without the iron mordant. In no instance have nuclei entirely devoid of chromatin been found. The vesicles in Figs. 13, 14, 17, Pl. II., and most of them in Fig. 12, are empty. If the interpretation offered is correct, then there must be a very large amount of chromatin that leaves the nucleus in Fig. 12. The meaning of the large flask-shaped vesicle attached to the female pro-nucleus in Fig. 15 is not understood.

There is some question as to whether this process takes place in all of the eggs preparatory to cleavage, but that it is very common and appears in well fixed eggs there can be no question. On the same slides were found mitotic figures preserved in all of their parts. The slides showing many of these phenomena were examined by Mr. George T. Hargitt who is at work on a similar problem at Harvard University and he confirmed the correctness

of these observations. The conditions certainly exist as drawn but more drawings could be made easily showing different relations of the chromatin masses and vesicles. The eggs just after deposition do not show these chromatin changes. After a time the chromatin that remains in the male and female pro-nuclei increases in amount and takes a deeper stain until the chromatin appears like that shown in Figs. 16, Pl. IV., and 19, Pl. III. Hargitt in Fig. 48 has two similar vesicles.

The vesicles last but a short time and usually are indistinguishable by the time that segmentation begins. But when such conditions as shown in Fig. 16 exist in the presence of a small vesicle containing chromatin near the female pro-nucleus and a second one between the two pro-nuclei, these remain longer in the egg. What their influence on subsequent development is, some light may be gained by a study of Fig. 49 (Hargitt). In this drawing there is a clearly defined mitotic figure with faint chromosomes; and a short distance away, three asters and their connecting fibers are around several vesicles. This Fig. 49 seems to be a later stage in the transformation of some of these vesicles containing chromatin and indicates a pseudo-segmentation in that it is not preceded by the fusion of the male and female pro-nuclei. It hardly seems as if such conditions played an important part in the future segmentation.

Fertilization in the Egg of *Pennaria*.

The spermatazoa penetrates the egg of *Pennaria* frequently before deposition, but the penetration is usual after the egg is laid. The false membrane may contain a large number of sperm heads that were unable to gain admission into the egg. The sperm head immediately after penetration becomes transformed into a vesiculate body, the male pro-nucleus. No definite place of entrance, nor path through the cytoplasm, nor the presence of asters could be determined. It remains smaller than the female pro-nucleus and is surrounded by fine cytoplasmic granules. Figs. 15, Pl. IV., 16, Pl. III., and 18, Pl. IV., give a good idea of the male pro-nucleus.

Figs. 17 and 19 show the approach of the pro-nuclei. The fine cytoplasmic granules surrounding the two bodies have united

in Figs. 15 and 19 and may have a stellate outline. It was practically impossible in Fig. 19 to determine whether there was a fusion at this stage of the two nuclei or whether they were merely in contact.

Polyspermia. — In Fig. 20, Pl. IV., there are shown conditions which seem to point strongly to polyspermia. The female pro-nucleus is present and some distance away a single vesicle which is similar in every respect to the male pro-nucleus of later stages. The cytoplasm is indented just opposite as if modified by the recent penetration of the sperm. Then close by this vesicle are nine other vesicles, somewhat smaller but otherwise identical with the single one. There is likewise the same modification of the cytoplasm just opposite. The natural explanation seems to be to regard these all as transformed sperm heads, and consequently polyspermia. Fig. 21, Pl. III., shows a second case of probable polyspermia where there are three nuclei, one much larger which is probably the female pro-nucleus. The other two are interpreted as male pro-nuclei. That several nuclei may exist previous to the first segmentation like this Fig. 21 and Fig. 48 of Hargitt there can be no question and that they are in some instances due to polyspermia I feel equally certain. But that this is the exclusive interpretation I do not accept because nuclear division may outrun cytoplasmic cleavage which results in giving several nuclei in the cytoplasm as Figs. 43 and 43*a* of Hargitt's clearly indicate and I have confirmed. It seems more probable that both explanations should be used in interpreting the multiple nuclear conditions.

Cleavage of the Egg in *Pennaria*.

This part of the paper on cleavage tends to show the manner of cleavage rather than a detailed description of the process as this has already been done by Hargitt. As was described in the section on fertilization, the two pro-nuclei approach preparatory to the first cleavage. Fig. 22, Pl. II., shows the prophase of the first cleavage. The female pro-nucleus has become much elongated and there are distinct asters at each end. The spindle fibers are just forming and the chromatin is still in the loose stage; the chromosomes are yet to form. The male pro-nucleus,

such of it as appears in the section, is near one end of the female pro-nucleus and much smaller. In Fig. 23 is shown another form of the prophase. This drawing was taken from the beginning of the second cleavage. The small prophase spindle lies entirely within the centrosphere and the chromosomes are in the form of vesicles. That these vesicles are modified chromosomes is proved by the condition of the chromosomes at the opposite pole of the old spindle where the process was not as advanced. Here some of the interzonal fibers were still in contact with the partly transformed chromosomes. The successive cleavage is so rapid in this instance that the new spindle has formed before the vesicles have united into a single vesicle, the nucleus. Nevertheless a normal spindle will result when the chromosome vesicles are transformed again into chromosomes. It is the study of such changes as these in the chromatin that convinced me that the vesicles in Fig. 10 were modified chromosomes and the vesicles in Figs. 12–16 were formed through the influence of chromatin astray in the cytoplasm. A third form of the prophase is quite common, Fig. 24, Pl. IV., where the nucleus is much elongated or somewhat irregular in outline. This prophase stage is farther advanced than the two previously described. The asters are larger and the forming spindle fibers more pronounced at each end. The chromatin has begun to take a deeper stain. In some respects all of these three forms of prophase are different, but the differences are not fundamental, and plenty of similar variations are known in other animals. They are all unmistakably by the mitotic process.

The metaphase is as typical as exists in any mitotically dividing cell. The chromosomes split and move to each pole, Figs. 23, 26, 27, Pl. II. During the late anaphase and early telophase the chromosomes become transformed into vesicles, Figs. 27, 23, may or may not unite into a single vesiculate nucleus before the next cleavage. I have been able to trace the nucleus continuously from its state in the unmaturated egg through all of the cleavage states. At no times does it dissolve other than in the normal mitotic changes. At no time does the total contents of the nucleus become dissipated throughout the cytoplasm to reform into separate nuclei. Other than the chromatin changes just

previous to segmentation, the nuclear phenomena in *Pennaria* appear perfectly normal.

Sometimes the cleavage is very regular as text-fig. 5 indicates, each cell shows the nucleus in the prophase and apparently so placed as to give off cells in the clockwise directions. The tilting of these nuclei and the perfect regularity is so apparent that this drawing might have been taken from an annelid or molluscan embryo, but in this same group of cells there was a most irregular embryo so that this regular condition is nothing more than an accident and is the only regular embryo among many sections.

The Centrosphere in the Egg of *Pennaria*.

It is difficult to decide on any of the old terms to describe the conditions in *Pennaria*. If the term centrosphere may include sphere and centrosome, the latter being potentially present only, then this may be taken as an acceptable term.

If there is a centrosome present in these eggs then it is an unstable body which varies greatly and is recognized with much difficulty. In all cases of the prophase, there remains a small clear area from which the fibers radiate but into which they do not penetrate. Fig. 24, Pl. III., shows a few fine granules in this area but their size, number, and staining reaction does not indicate that they are at all constant, nor can one detect that there is any inherent relation between these granules and rays or spindle fibers. That there is probably some substance that plays the part of a centrosome is apparent in Fig. 23, Pl. III., where the whole new spindle lies entirely within the old centrosphere. Therefore, it seems as if one might say that certain of these granules have potential centrosome properties and are possibly in the way to become differentiated; that is to say, that the elaborate centrosomes of mollusca for example indicate a higher degree of differentiation, while in these hydroids a similar result is produced by several granules which cannot be differentiated from the rest of the sphere substance.

The transformation of this centrosphere keeps pace with the changes in the chromosomes. By the time that the anaphase is reached the centrosphere substance has increased greatly in size,

Figs. 25, 26; and in late anaphase is very conspicuous, Fig. 27. After the metaphase stage, the astral fibers are mostly composed of granules, Figs. 25, 26. The interzonal fibers persist for some time and are frequently bent as the cleaving cytoplasm passes through them. At such times, the chromosomes lie at each end of a V-shaped figure. As the fibers disappear and the chromosomes become transformed into a nucleus, the centrosphere substance largely becomes an indistinguishable part of the cytoplasm, but a small portion remains as a clear, narrow region surrounding the nucleus. This means that the deeply staining, newly formed "resting nucleus" with a narrow transparent area around it in cleavage is perfectly normal, being the last remains of the centrosphere.

Papillæ in *Hydractinia* and *Pennaria*.

Hargitt (p. 469) has described these as ectosarcal phenomena. My study confirms his and shows that in both of these hydroids the papillæ are common in the unsegmented egg and have even been seen while the egg was in the medusa. There is no particular region on the egg where they arise. At first these papillæ were regarded as polar bodies, especially in *Hydractinia*, but when they were found on the vegetal pole and the female pro-nucleus was present at the animal pole in the same egg, such an interpretation was impossible. I believe that these papillæ are what Miss Bunting saw and described as polar cells, which is a mistake that might be easily made. In *Pennaria* these papillæ push the false membrane away from the egg as they form and after being set free remain in this same structure for some time. The papillæ in both species are wholly devoid of chromatin and so far as could be determined entirely cytoplasmic phenomena.

Fragmentation of the Nucleus, — Amitosis.

Fragmentation. — If by fragmentation of the nucleus is meant that the entire nucleus disappears and its contents disperse throughout the cytoplasm then I find no evidence of such a process in these hydroids. But what shall be said of the chromatin changes before maturation in *Hydractinia* and in *Pennaria* after maturation where large quantities of chromatin migrate into

the cytoplasm never to return to the nucleus so far as one can determine? This certainly seems to be a kind of fragmentation. Why does it occur? Has the maturation phenomena failed to fully prepare the egg nucleus for fertilization? These are questions which those who would make the development of all eggs conform to a definite series of changes must explain.

Amitosis. — By this process it is understood that the nucleus divides without the chromatin passing through a complicated series of changes and without the formation of a spindle. Frequent search has been made for amitosis in these eggs but without finding any positive evidence. The irregular and claviculate shaped nuclei were critically observed and in every instance eventually either asters or the very characteristic chromatin changes were taking place in them. The mere shape of the nucleus in *Pennaria* is no indication of amitosis, nor is it necessary that the chromosome vesicles become transformed into the single "resting nucleus." *The cluster of vesicles which Hargitt frequently finds is not uncommon in my material but is interpreted in this paper as late telophase.* This is a point concerning which there may be a difference of opinion but taking all of the facts into consideration, these vesicles seem to me to indicate mitosis rather than amitosis.

Inclusions in *Pennaria*.

There are found in the eggs of *Pennaria*, even before maturation, bodies which for the lack of a better term are designated inclusions, Fig. 29, Pl. IV. Thus far they have not been seen in the segmenting egg. As many as three such inclusions have been discovered in a single egg. When newly formed, the substance of the inclusion takes the same stain as the surrounding cytoplasm, but in the older stages this contained substance stains faintly, eventually leaving a cavity. This cavity then is obliterated by the encroachment of the cytoplasm. The substance within the inclusions in appearance and staining reactions is certainly similar, if not identical, to the cytoplasm, and the whole structure looks like a food-vacuole in which cytoplasm is being digested. Their origin has not been determined. They can hardly be regarded as polar bodies, because they may appear before maturation begins.

Summary.

1. In many particulars this work is a confirmation of the previous paper by Hargitt, especially in regard to the several nuclei in the unsegmented egg, the irregular shape of many such nuclei, and the irregular phases of cleavage.

2. The ova arise in any region of the polyp, which is contrary to Bunting's statements. The young ova gradually increase in size, during which time the nucleolus becomes vacuolated and the cytoplasm is occupied by numerous microsomes which become transformed into spherules. The cytoplasm changes its staining reaction during this time.

3. The chromatin during the growth of the egg stains less intensely than when in the younger state. Much of the chromatin migrates into the cytoplasm and is surrounded by vacuoles. The highly vacuolated condition of the cytoplasm is probably directly due to this migrating chromatin. The size of the nucleus decreases greatly.

4. In *Hydractinia* there were found three distinct kinds of granules, yolk masses, coarse granules and small bodies around the periphery. The small granules are distributed exclusively to the ectoderms.

5. Maturation begins in *Hydractinia* before the eggs are deposited. The process is by the formation of a distinct mitotic figure. It is very rare to find a polar body attached to the deposited egg.

6. The female pro-nucleus is very much smaller than the egg nucleus before maturation but it persists as a definite structure until cleavage begins. It is not at any time indistinguishable. The male pro-nucleus moves through the cytoplasm until it approaches the female pro-nucleus when the two fuse and fertilization is effected.

7. The first, and all subsequent cleavages, is by the mitotic process. A definite segmentation cavity is formed in the two-celled stage which increases in size. This cavity is gradually filled with cells until the planula is a solid mass of cells.

8. The false membrane in *Pennaria* is a transitory structure and probably of a fluid nature. Later its place is taken by a true membrane.

9. Maturation in *Pennaria* begins before the eggs leave the medusa. The polar bodies are ephemeral in character and rarely found attached to the deposited egg. The polar bodies are formed by the mitotic process.

10. After the two polar bodies are formed in *Pennaria* there is a distinct migration of a considerable amount of chromatin into the cytoplasm. The chromatin is transformed into vesicles which eventually are taken up by the cytoplasm. Sometimes these vesicles contain chromatin and persist for some time and may (?) divide mitotically, giving rise to a pseudosegmentation.

11. The spermatozoa may penetrate the egg before it is laid. The sperm head is transformed into a male pro-nucleus which moves through the cytoplasm toward the animal pole to unite with the female pro-nucleus.

12. Cleavage in *Pennaria* is at all times by the mitotic process. The chromosomes become transformed into vesicles during the late anaphase and early telophase. The vesicles may or may not unite into a definite "resting nucleus" before the next cleavage.

13. During cleavage in *Pennaria* there is a distinct centrosphere which contains granules with centrosome powers. This centrosphere is more conspicuous in *Pennaria* than in *Hydractinia*. The new prophase spindle arises within the old centrosphere.

14. Papillæ are found in both *Hydractinia* and *Pennaria* before segmentation, much as described by Hargitt.

15. A partial condition of fragmentation is seen in the migration of chromatin into the cytoplasm in both species.

16. No clear evidence of amitosis was observed.

17. Inclusions are frequently found in the egg of *Pennaria*.

October 20, 1908.

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POSTSCRIPT.

CHAS. W. HARGITT.

As supplemental to the foregoing paper it may not be amiss to add a few brief notes and comments. First, to the effect that it comprises an integral phase of work which has engaged the writer for many years, and which is still in progress. This particular feature was undertaken at my solicitation in the summer of 1906, as stated by the author. Second, at the same time I likewise turned over to my son, G. T. Hargitt, material for work along similar lines, a brief report of which has already been made. (*Science*, March 12, 1909.)

Smallwood's paper was completed nearly a year ago, but was at my request delayed, pending completion of further work of my own which was well advanced, and which it was intended should appear at the same time as intimately related thereto. The appearance of a brief note by Beckwith (BIOL. BULL., March, 1909), suggests the desirability of the issue of Smallwood's paper without further delay.

Aside from one or two points suggested by Beckwith I shall not undertake any detailed comments in this connection. Her rather matter-of-course dismissal of my presumed errors as to maturation with the remark that it was due "simply to the fact that eggs were not collected at the right time of day" is, to say the least, somewhat gratuitous! One does not usually follow in-

vestigations over a period of ten years without having taken *some* precautions against the more obvious sources of error. The fact is, I had long ago provided against that contingency. Again, her equally hasty dismissal of any question of methods of technique is without warrant. It was this more than any other one matter that proved an obstacle to satisfactory cytologic results. This I have called attention to in at least two of my more recent papers. And the precaution mentioned in the above paper by Smallwood as to this point was explicitly my own suggestion.

A brief comment as to the question of amitosis raised by both Smallwood and Beckwith must suffice for the *present*. In the first place I have never questioned the fact of mitosis in any of the cases under review, as the most cursory attention to my papers will show. Whether there be *amitosis* is purely a question of fact. Were my own results the only evidence it might very well be questioned. Facts adduced from almost every phylum of the animal kingdom are too well known at present to warrant further dogmatism on *a priori* or theoretical grounds. Whether my interpretation of the significance of the nuclear and chromatin fragmentation and the vesiculate "nuclear nests" may be warranted I shall defer for later consideration.

EXPLANATION OF PLATES.

Explanation of Plate I.

Fig. 1. Egg nucleus with small amount of cytoplasm to show the migration of the chromatin. *Nl'*, nucleus; *Nl'*, nucleolus; *chr*, chromatin.

Fig. 2. Portion of the cytoplasm and nucleus. The chromatin is mostly in vacuoles. *Nl'*, nucleus; *chr*, chromatin.

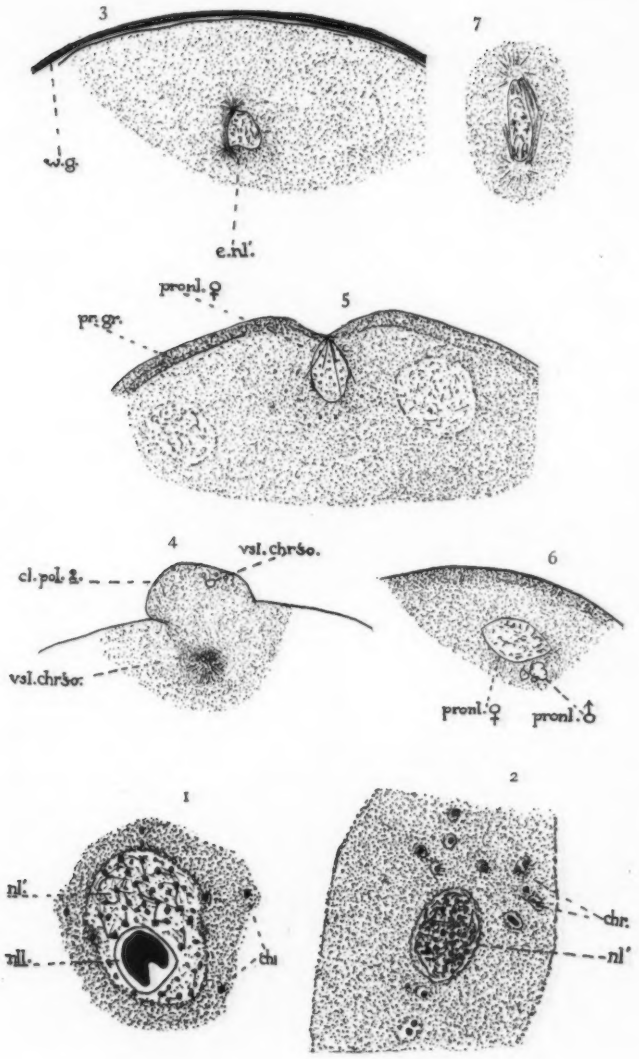
Fig. 3. Prophase first maturation. The walls of the nucleus are still intact. The egg is within the gonophore. *E. Nl'*, egg nucleus; *w. g*, wall of gonophore.

Fig. 4. Telophase of the second maturation. Egg deposited. *Cl. pol*^{2}, second polar body; *vsl. chr'so*, chromosome vesicles.

Fig. 5. Female pronucleus showing the remains of fibers that have persisted. Pronl. ♀, female pronucleus. *pr. gr*, peripheral granules.

Fig. 6. The approach of the male pronucleus. The female pronucleus is in the early prophase. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

Fig. 7. Prophase of third cleavage shows an elongated nucleus with asters and forming spindle fibers.


3
w.g.
e.nl'.
7
pronl.♀
pr.gr.
5
4
cl.pol.♂
vsl.chrso.
vsl.chrso.
6
pronl.♀
pronl.♂
1
nl'.
nll.
chi
2
chr.
nl'

Explanation of Plate II.

Fig. 8. Is taken from one of the cells shown in Fig. 9. The vacuolated condition of the cytoplasm is shown in the clear spaces. The spindle is in the anaphase and so directed as to set a cell free in the segmentation cavity. There is a large centrosphere at each pole.

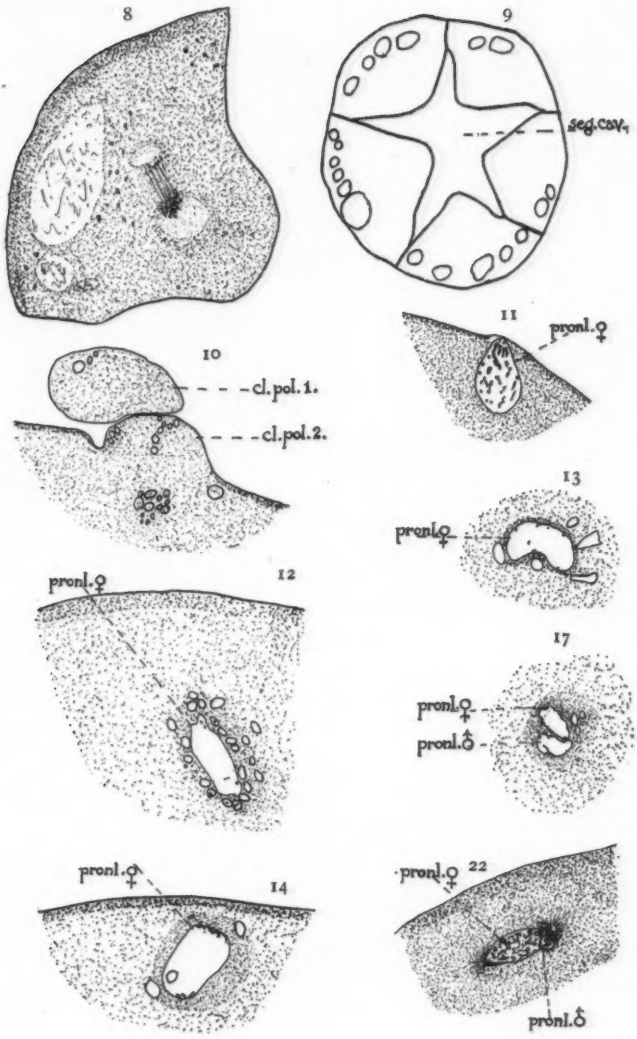
Fig. 9. Outline drawings of embryo. Numerous vacuoles are present in the cells. *Seg. cav*, segmentation cavity.

Figs. 10 to 29 are from *Pennaria*.

Fig. 10. Telophase second maturation. Egg in medusa. *Cl. pol. 1*, first polar body; *cl. pol. 2*, second polar body.

Fig. 11. Female pronucleus drawn from an egg before deposition. Pronl. ♀, female pronucleus.

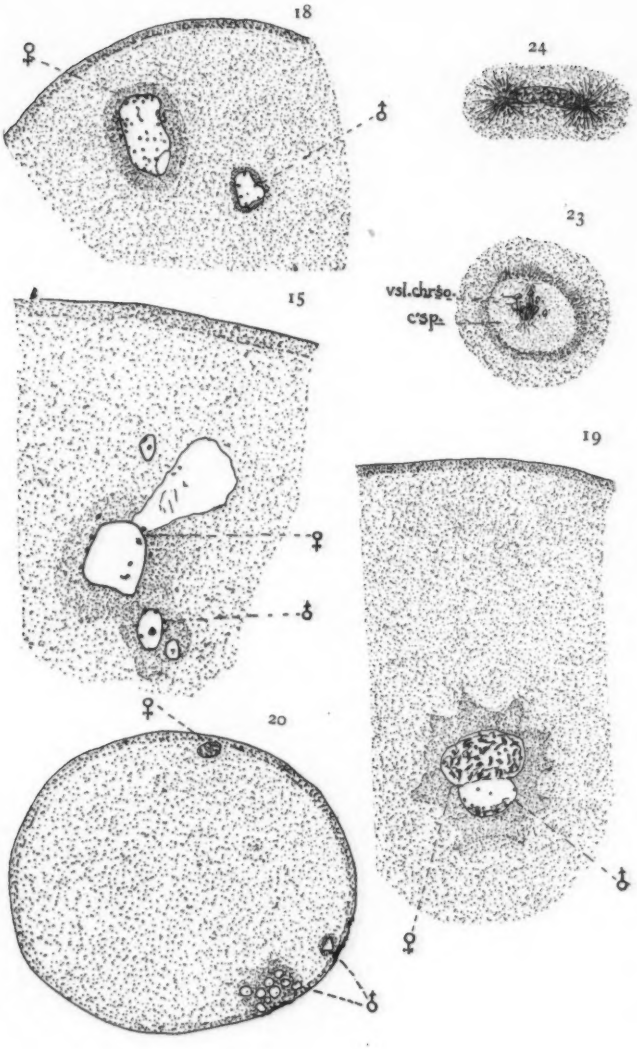
Figs. 12 to 18 show the migration of the chromatin from the pronuclei into the cytoplasm. Pronl. ♀, female pronucleus; ♂, male pronucleus.


8
9
seg.cav.
10
cl.pol.1.
cl.pol.2.
11
pronl.♀
12
pronl.♀
13
pronl.♀
17
pronl.♀
pronl.♂
14
pronl.♀
22
pronl.♀
pronl.♂

Explanation of Plate III.

Fig. 19. Union of the male and female pronuclei. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

Fig. 20. Low power drawing of entire egg. The fine peripheral granules extend clear around the egg. Several nuclear-like bodies are interpreted as polyspermia.


18
24
23
vsl.chrs.
c'sp.
15
19
20

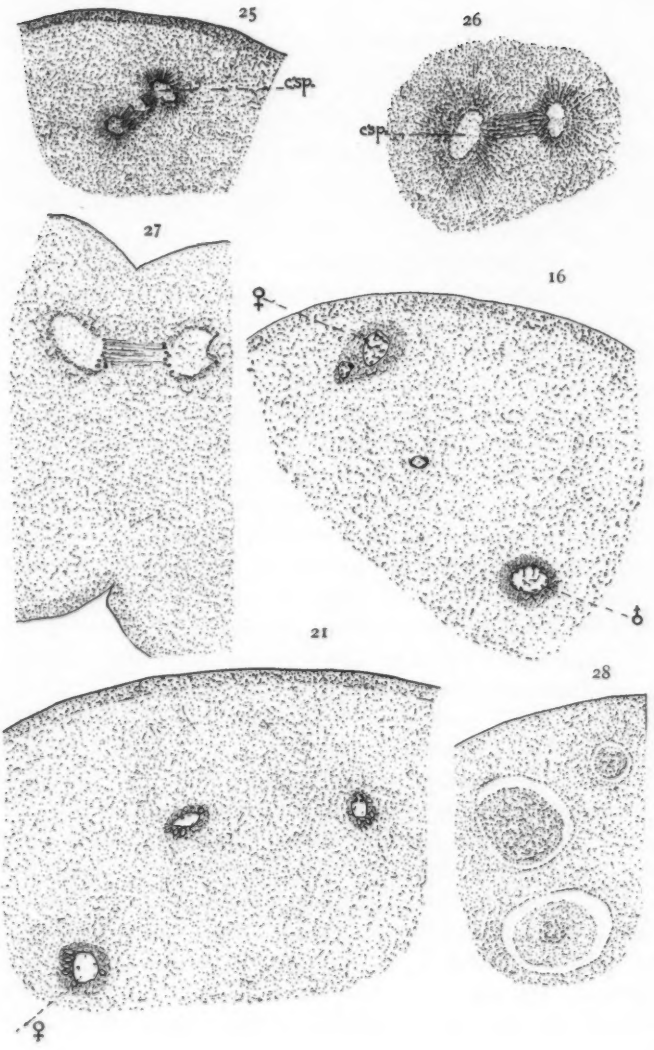
Explanation of Plate IV.

Fig. 21. Shows the presence of several nuclei in the unsegmented egg, one of which is probably the female pronucleus, pronl. ♀.

Figs. 22 to 24. Three forms of the prophase in cleavage. Fig. 22, conjugation of pronuclei and formation of first segmentation spindle. In Fig. 23 the chromosome vesicles have not united. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus; *vsl. chr'so*, chromosome vesicles; *c'sp*, centrosphere.

Figs. 25, 26, 27. Show the appearance of the segmentation spindle, migration of the chromosomes, and presence of the centrosphere; *c'sp*, centrosphere.

Fig. 28. Inclusions in the cytoplasm.


25
csp.
26
csp.
27
16
♀
♂
21
28
♀

NEW OR LITTLE KNOWN PERMIAN VERTEBRATES. PARIOTICHUS.[1]

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The University of Chicago Expedition to the Permian of Texas during the autumn of 1908 was very fortunate in finding a skeleton of a small reptile enclosed in a nodular matrix, probably the most complete of any specimen hitherto obtained from that formation. It is of especial interest since it gives, for the first time, a natural skeleton of a cotylosaur with all its bones in anatomical relations, scarcely a single one disturbed by extraneous force in fossilization. The original nodule measured about six inches in its greater, by five in its lesser diameter, and about two inches in thickness. The nodule, as discovered, was split horizontally, the thicker portion enclosing most of the skeleton lying upon its back; the thinner with portions of the bones partly enclosed in it, and with the right front leg almost wholly so. One small piece of the thicker side, and a yet smaller fragment of the thinner were not recovered. The missing portions, however, are of minor importance, and are in part supplemented by the two blocks. The specimen was discovered on a gently sloping surface near the Wichita River, north of Mabelle, by Mr. Paul Miller.

The material of which the nodule is composed is a rather hard argillaceous limestone, and has necessitated very patient labor on the part of Mr. Miller, with awl and needle, in its preparation, many of the bones being so small as to require the use of a magnifying glass. The skeleton, which measures nearly fifteen inches in length, is closely coiled, the tip of the tail lying under the front extremity of the skull. As exposed on the two blocks, the hyoid bones are in place; the pectoral girdle is very slightly displaced, with both arms articulated; the right arm is strongly flexed at the elbow, with its outspread hand underlying the pos-

[1] "Cotylosauria," *Journal of Geology*, XVI., p. 139; *Lysorophus*, this journal, XV., p. 229; *Diplocaulus*, *Trans. Kansas Acad. Science* (in press); *Trematops*, *Journal of Geology*, XVII. (in press).

terior part of the right mandible; the left arm is extended backward close to the vertebral column, its fingers protruding from the edge of the block and for the most part lost; both hind legs

FIG. 1. *Pariotichus laticeps* Williston. Photograph of specimen, from the thicker half of nodule; natural size.

are articulated throughout, turned backward by the side of the tail, and not a single bone seems to have been lost or disarticu-

lated; both the feet, unfortunately, are lying in part upon their fibular side, concealing some of the bones; the tenth to the thirteenth caudal vertebræ are disarranged and partly missing, probably due to the fact that they lie partly over the right foot; the small terminal vertebræ of the tail are also missing, where

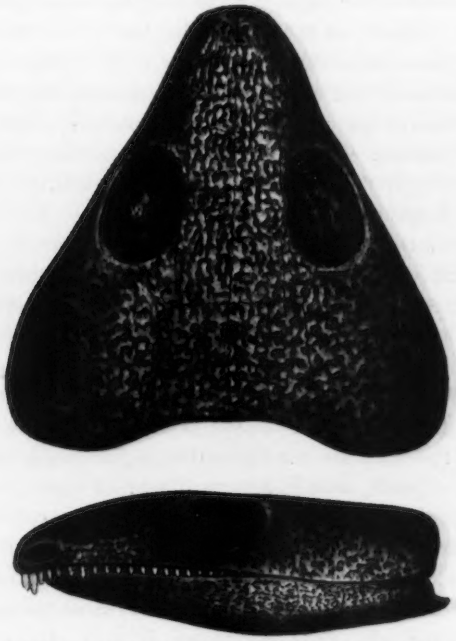

FIG. 2. *Pariotichus laticeps*, skull from above and from the side; natural size.

they protruded from the margin of the slightly eroded nodule. On the whole, the only doubtful details of the skeleton are the number and arrangement of the tarsal bones, and the extreme tip of the tail. The extreme terminal phalanges of the first three fingers, because of their minute size, may have been destroyed in the preparation of the block, or slightly dislodged.

Skull (Figs. 1, 2). — The skull is in a marvellously perfect condition, the only injury it has suffered being a very slight erosion of the extreme tip of the muzzle where it protruded from the edge of the nodule. It is remarkable among reptiles for its

great width and depression, its width posteriorly being fully equal to its length. The pitting of the upper surface is small and reticulate, with a slight, though distinct, indication of longitudinal ridges. The parietal and frontal bones posteriorly are slightly concave in the middle. The nares are small, situated nearly at the extremity of the muzzle, oval in shape and directed more outward. In front of the orbits the face is a little constricted, with the sides more strongly convex. From the front of the orbits the lateral lobe of the cranium is convex outward to about midway between the orbit and the hind border, where the curve is slightly inward. There is a rather strong emargination of the hinder border of the cranium in the middle, with the outer third on each side gently convex or nearly straight. The large orbits are located a trifle in front of the middle of the skull. They are oval in outline, with the long diameter antero-posterior, measuring eighteen millimeters in length by fifteen in breadth. The plane of their margins is directed a little forward and upward at an angle of about 45°. The large pineal foramen is nearly midway between a line drawn through the posterior margin of the orbits and the hind border of the skull in the middle line. The sutures of the skull are, for the most part, indicated by very delicate lines, requiring a hand lens to follow. I have given such as I feel sure of. The plane of the upper surface of the skull is nearly horizontal as far forward as the front end of the orbits, whence it slopes gently to the front extremity, with a slight convexity. The mandibles are in position on the under side, slightly pushed to the left. The symphysis is short, the rami rather narrow in front, their outlines very much like those of the side of the cranium, curving inward posteriorly. They are broadest and deepest a little back of their middle, or opposite the transverse bones, which abut against them. Distinct sutures for the splenial, dentary and angular are seen anteriorly on the outer side. The dentary ends by a broad curve upward, with the slender prolongation of the angular enclosed between it and the margin of the splenial below. At the hind extremity the small, inwardly curved angular process is visible. The slight lateral pressure upon the mandibles has left exposed the insertion of the maxillary teeth of the right side, but the very delicate teeth them-

selves have suffered in the preparation, though their roots are clearly to be seen. On the premaxillary there are three stout teeth, the largest of the series of either jaw. Of these the third is the smallest, the first and second of nearly equal size, judging from their roots. Back of the premaxillary teeth, in addition to the roots seen on the right side, the teeth themselves are preserved on the left side, crowded rather closely upon the mandible. They are all rather small, the fifth or sixth of the series, counting the premaxillary teeth, about a third of the distance to the orbit back of the narial opening, is the largest. They are all rather obtusely pointed, and are separated by spaces about equal to or somewhat less than the width of the teeth themselves. In a space of ten millimeters back of the largest maxillary tooth there are five teeth. The mandibular teeth cannot be made out. It is altogether probable that there are additional teeth in secondary rows upon the maxilla and dentary, but the close union of the mandibles with the cranium prevents their detection.

The palate is very fully exposed and is undistorted. The internal nares, placed far forward, are above the mandibles and yet concealed by the matrix. The broad, flat palatines and vomers — for sutures are nowhere determinable — diverge very gradually to about midway between the mandibular symphysis and the basisphenoid, where they separate more widely, leaving a rather large ovate space, yet filled in with matrix. I can distinguish no presphenoid in this ovate space, but it is possible one exists directed obliquely toward the roof of the skull. Along the margin of these bones by the side of the ovate opening and anteriorly are one or two rows of minute tubercular teeth, and, just in front of the descending convexity of the transverse, there is an oblique row of six or seven palatine teeth on each side. The lateral palatal plates descend posteriorly in a convex surface, to nearly the lower margin of the mandibles, with a thinned, convex posterior margin. The lower convexity of these transverse bones (for they are doubtless separate elements, though they have never been suturally distinguished) is covered with a patch of tubercular teeth. The union of the pterygoids with the basisphenoid is very evident in the constriction at either side of the front of the basisphenoid, but the suture is not determinable. From this con-

striction and union with the short basipterygoid processes of the basisphenoid, the posterior plates of the pterygoids diverge to the inner side of the quadrates, opposite the inner angle of the articular bone of the mandible. These plates have a thin, straight, horizontal lower margin, whence they slope inwardly and upwardly into a somewhat concave surface, narrow and nearly vertical in front, widened behind, leaving a small oval space anteriorly between them and the basisphenoid. The basisphenoid is narrow in front, gently widened behind, shallowly concave in the middle and limited on either side by a rather rugose ridge. From near the posterior, somewhat divergent, ends of these ridges, a slender bone runs outward and backward by the side of the inner margin of the pterygoid plate. It is in all probability the stapes. Upon the whole, the structure of the palate and occipital region is quite like that of *L. hamatus*, which will be fully described and illustrated in a future paper from a remarkably perfect specimen in the University collections. Lying under the palate were two slender rods. One of these has been necessarily destroyed in getting to the palatal surface; the other still remains in the matrix. They nearly meet in the middle anteriorly, just back of the interpterygoid vacuity, diverging posteriorly nearly parallel with the margins of the pterygoid plates to near the quadrate. Back of these and articulated with them are a pair of more slender bones bent inwardly near their middle, and terminating acutely behind, nearly parallel with the front margins of the clavicles.

Vertebræ. — The vertebræ are united in a continuous series from the skull to near the tip of the tail, forming a U-shaped curve. The pectoral girdle still conceals the anterior five or six of these and the posterior seven of the presacral series were lost in the missing fragment of the nodule. However, the top of the nodule over these missing seven vertebræ has preserved in part impressions of them, and, inasmuch as the vertebræ themselves, where exposed, are all of precisely the same length, seven and a half or eight millimeters, the number of presacral vertebræ in the entire series is determinable with but slight chance of error. This number was either twenty-three or twenty-four. Broili has determined the number of presacral vertebræ in *Labidosaurus hamatus* as either twenty-four or twenty-five; twenty-three or possibly

twenty-four is the number of presacral vertebræ in the rhachitomous *Trematops* as determined by me; twenty-three or twenty-four was the number ascertained by Thevenin in *Sauravus costei*, a probable reptile from the Upper Carboniferous of France; and I believe that *Isodectes Copei*, from the Lower or Middle Mississippian of Linton, Ohio, had the same number. This uniformity seems to be more than a coincidence. Of this number the atlas alone can be called, as in the amphibians, a true cervical. All the vetebræ bore ribs, and, inasmuch as there was no sternum in these early forms, a distinction into neck and trunk is impossible. The centra of our specimen are rather slender — more so than in *Labidosaurus* — each with a marked constriction in the middle. Some of the posterior vertebræ lie partly upon their sides, disclosing the attachment of the ribs. No distinct process is seen on the centrum for them, differing in this respect from *Labidosaurus*, though doubtless the lower part of the proximal extremity of the ribs did articulate with the centrum. A small intercentrum is present in one or two of the anterior vertebræ, and a small space is seen between the lower edges of the centra of all. The sacral vertebræ are of course not visible in the pelvis. There were, in all probability, but two sacral vertebræ, though this is not certain since another form of Permian reptile, which will be illustrated and described later, has three, while *Labidosaurus* has but two. If the vertebræ above the pubes and ischia are of the same length as those immediately following or preceding, there are six concealed from view, one of which is the first presacral, and three the basal caudals. Behind the pelvis eight caudal vertebræ in a connected, gently curved series are visible. They are somewhat shorter and more slender than the presacral vertebræ, the entire series measuring about forty-six millimeters. Beginning with the second of these there are long, slender chevrons, each reaching to beyond the end of the next succeeding centrum, that is, about fourteen millimeters in length. Just how far back these chevrons continue is not certain, but at least eight centra bore them. The first entire vertebra back of the pelvis bears a long, curved rib on each side, springing from the anterior end of the centrum. The next two vertebræ doubtless have similar, but short ribs, though only the

proximal ends of them are seen. I do not think that the following vertebræ bore ribs, or, if so, they were much shorter. I can find no indications of such. In the restoration, these caudal ribs are shown directed backward, as in the matrix. Doubtless in life they were directed more downward, indicating a thick basal portion of the tail, probably compressed from side to side. Beyond the eighth visible caudal there is a break in the series, the only indication of contemporary extraneous force shown in the entire skeleton; and this may have been due to the fact that this part lies somewhat under the right hind leg in the nodule — that is, if the skeleton was originally fossilized in a prone and not supine condition. Following this gap there are three articulated vertebræ in line with the curvature of the basal portion of the tail. They are more slender than the preceding ones and clearly lie in their original position, the intervening five vertebræ having been dislodged, fragments of which are still preserved near the break. The extreme tip of the tail came near the margin of the nodule, at or below the tip of the muzzle, and has been destroyed by the erosion of the nodule. Perhaps a half dozen very small vertebræ are missing here, making altogether about twenty-five vertebræ for the number in the tail, or for the entire column about fifty, with a possible error of three or four more.

The dorsal ribs have an expanded proximal end, but without distinct differentiation into head and tubercle. The first one visible is attached to the vertebra concealed in part by the caudal end of the interclavicle, that is, the seventh or eighth of the series. It is altogether probable that the first four or five ribs were shorter, with expanded distal ends, as in *Labidosaurus*. From the eighth to the seventeenth the ribs lie nearly parallel on the right side, on the left being crowded more together at their ends. The longest of them measure forty-six millimeters along their considerable curvature. They are slender throughout. The broken ends of the eighteenth and nineteenth are visible in the matrix, but little if any shorter than the preceding ones. A fragment of what should be the twenty-first or twenty-second is also visible on the right side, but its length is not determinable. I have, therefore, shaded the last four ribs in the restoration, and it is possible these vertebræ were entirely ribless, as I have decided they were in *Labidosaurus*.

Pectoral Girdle. — The pectoral girdle lies almost perfectly in position, the hind end of the interclavicle only, turned slightly to the right, and the right coraco-scapula pushed slightly forward, or the left one backward. It is very certain that the girdle was attached immediately back of the skull, the front part underlying the occipital condyle even. In structure it is almost identical with that of *Labidosaurus*, as figured by me. The position in which they lie has slightly separated the clavicles at their scapula, attachment. I find no indications of a cleithrum. It is very evident that the coracoids in life were in immediate contact along the median line, covered over by the prolongation of the interclavicle. The scapulæ curve upward at an angle of about forty-five degrees from the plane of the coracoids. Possibly this angle has been reduced slightly by pressure, but I think not. The scapulæ are directed, not backward, as has been supposed, but obliquely upward.

Front Legs. — The humerus is of the usual shape, expanded proximally and distally in planes meeting each other at an angle of sixty or seventy degrees. The bone, like all other parts of the appendicular skeleton, is distinctly more slender than in *Labidosaurus*. The radius is a rather slender bone, cylindrical at its proximal, flattened and expanded at its distal extremity. The ulna, much broader and thicker at its proximal end, has a distinctly produced olecranon, and the curvature of the rather slender shaft in the middle is distinctly away from the radius. The most of the forearm and foot of the right extremity are preserved in the thinner block, the proximal ends of the radius and ulna in the thicker close by the right pubis. The hand, as thrust forward below the right mandible, is outspread and fully articulated, the middle of the wrist somewhat depressed by the angle of the mandible in the mud, slightly turning the distal end of the ulna. The terminal phalanges were probably present or but slightly dislodged, but their minute size has made it almost impossible to detect and work them out from the hard matrix. The carpus clearly agrees in its chief features with the carpus of *Labidosaurus "incisivus,"* as figured by Case and myself, save that the parts were reversed in the figures. I give herewith a better figure of the labidosaur carpus, which has been more completely removed from

its matrix (Fig. 4). Because of the compression of the middle part of the carpus in our specimen, it is impossible to be quite sure of the presence of both centralia. The radiale is much broader than long, articulating with the radius, the distalia of the second and third digits, and with one or possibly two centralia. The ulnare is a much larger bone, articulating proximally with the ulna, distally with the two inner distalia, and on the outer side with the

FIG. 3. *Pariotichus laticeps*. Photograph of right front foot, from thinner half of nodule; natural size.

intermedium. That there is a free intermedium here as in *Labidosaurus* is certain, but I cannot be quite sure of its extent, a part of it being apparently covered over by the radius. It articulates, as in *Labidosaurus*, proximally with the ulna and distally with a centrale. Five digits are present, as was to be expected. The first is represented by its metacarpal only, either slightly removed from its articulation with the radiale, or, what is more probable, with its distale lost, or cartilaginous. In the restoration it is shown removed from the carpal bones as in the photograph of the hand also given herewith (Fig. 3). The first metacarpal is the shortest of the five, and is only moderately ex-

panded distally. No phalanges are preserved. The second metacarpal is much longer than the first, and is much constricted in its middle. It has one short phalanx articulated with it, but little more than half the length of the metacarpal. Additional phalanges are not preserved, but, from its size, it seems very probable that two more, and not more than two, were originally present. The third metacarpal is much like the second, but is a little longer. Two phalanges are present, the first about two thirds the length of the metacarpal; the second fragmentary. There may have been a third, ungual phalanx present. The fourth metacarpal is the longest and stoutest of all, its proximal articulation more oblique than is the case with the preceding one. The first phalanx is about three fifths the length of the metacarpal. The second phalanx, much shorter and smaller, has at its tip a small fragment. There may have been a fourth phalanx, though there is not much probability of it. The fifth metacarpal is a little shorter than the fourth, somewhat curved and more slender. It has a small and short proximal phalanx and a fragment of a distal one at its tip. In all probability there were no more. It is, it is seen, impossible to say with certainty what the phalangeal formula of *Pariotichus* was, save that quite surely it was not that of the modern lizards and *Sphenodon*, 2, 3, 4, 5, 3. In much probability it was 2, 3, 3, 4(3), 2.

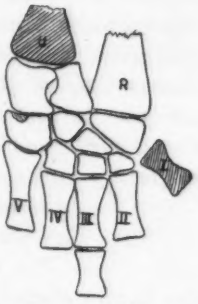

FIG. 4. *Labidosaurus*. Carpus; one half natural size.

Pelvis. — The under side of the pelvis lies in perfect preservation and position. It is, like the pectoral girdle, of the "old fashioned" type, elongate and plate-like, without thyroid foramen. The two sides meet in a long median symphysis, closely applied, but not sutural. The pubes, broadest in front, have a slight emargination in the middle in front. The small pubic foramen lies near the acetabulum, at the junction of the first and second thirds of the combined bone. Just inside this foramen, and a little to the outer side of the middle of each pubis, a pronounced thickening or ridge runs forward to the anterior margin, forming a

shallow fossa on each side of it. The pubo-ischial suture is, apparently, a little back of the pubic foramen, running transversely across. It is not at all distinct, and I am not sure of it. Lying in the median emargination of the pubes there are three or four very slender ventral ribs, and by their side, a fragment of what appears to be a thin plate, which may have articulated with the thickening of the front margin before mentioned, a thickening characteristic of all the smaller cotylosaurs, apparently. The pelvis, so far as it can be seen, is almost identical with that of *Labidosaurus*, and other Permian cotylosaurs, forms of which will be figured later.

Legs. — As stated, the hind legs are both preserved, trailing backward from the acetabulum, both of them somewhat bent at the knee. The femur of the right side suffered somewhat in the fracture of the nodule, and is only partly preserved. That of the left side, so far as it can be seen partly embedded in the matrix, agrees quite with that of *Labidosaurus*, though more slender. The tibia, also shown best on the left side, offers nothing unusual. The fibula, hitherto unknown in the cotylosaurs, is strongly curved, with a considerable expansion at its lower extremity, and with a small, rounded upper end. The feet, unfortunately, have both been preserved lying more or less on their fibular side, and in consequence with the toe bones more or less concealed. However, it is quite certain that no force was brought to bear upon them to displace them, save that of their own weight. The lower leg of the right side and the tarsus are spread out flatly, but with the digits piled upon each other. A large, flat fibulare articulates with the fibula of the right side in position, closely articulating on the inner side with another large bone, evidently the united tibiale and intermedium. Four tarsal distalia are visible. The shapes of the bones distinguished agree in general so well with those of *Labidosaurus* as figured by me, that I have no hesitation in giving the others from the same genus, shaded in the figure. The tibiale, however, must have been shorter than in *Labidosaurus*. As regards the toes, all five metatarsals are visible on one or the other side, and many of the phalanges, save those of the fifth toe. In the figure given in the restoration, the unshaded phalanges of the other toes are given precisely in the

positions they occupy with regard to the tarsus, so that the length of the toes is quite certain. Those phalanges which cannot be extricated from the matrix are shaded. In all probability the phalangeal formula is like that of the front feet; certainly there cannot be a greater number.

Six genera of Permian reptiles, all from Texas, are referred by Cope to the family Pariotichidæ; and, notwithstanding the difference in the teeth, I am disposed to add the seventh genus, *Labidosaurus*, to the same family. They are defined by Cope as follows:[1]

1. Teeth on the maxillæ and mandibles in a single series.....*Labidosaurus* Cope.
   Teeth in more than a single series.....2.
2. External nostrils inferior; mouth posterior in position; mandible short and with a few acute teeth.....*Hypnopous* Cope.
   External nostrils lateral.....3.
3. Palatal and splenial teeth with compressed crowns.....4.
   Palatal and splenial teeth obtuse, forming a grinding pavement; median maxillary and anterior incisor teeth enlarged.....*Pantylus* Cope.
4. Teeth equal, acute.....*Isodectes* Cope.
   Teeth increasing gradually in length anteriorly.....*Captorhinus* Cope.
   Teeth enlarged in the middle of the maxillary and anterior part of the incisor series.....*Pariotichus* Cope.

The genus *Helodectes*, provisionally placed in the Pariotichidæ, is distinguishable by the two rows of teeth on the jaws, the "bases of which are wide ovals, transversely placed."

Isodectes is figured by Cope as having the prefrontals and postfrontals meeting broadly over the orbits, widely excluding the frontals from the orbit. The skull, moreover, is much longer than broad in the type species, *I. megalops*. *Pantylus* also has the prefrontals and postfrontals meeting broadly as in *Isodectes* in the type species, *P. cordatus*, which, moreover, has a rounded muzzle, and is widely expanded posteriorly. *Captorhinus* has an elongate, pointed skull, with the orbits twice the diameter of the interorbital space. *Hypnopous* is wholly out of consideration because of the remarkable position of the nares. Assuming that our species has more than one row of teeth on maxillæ and mandibles, its exclusion from the labidosaurs is of course evident. As that character cannot be determined save by the mutilation of the otherwise perfect skull, the doubt must be left. By ex-

[1] *Proc. Amer. Phil. Soc.*, XXXIV., 1895, p. 445.

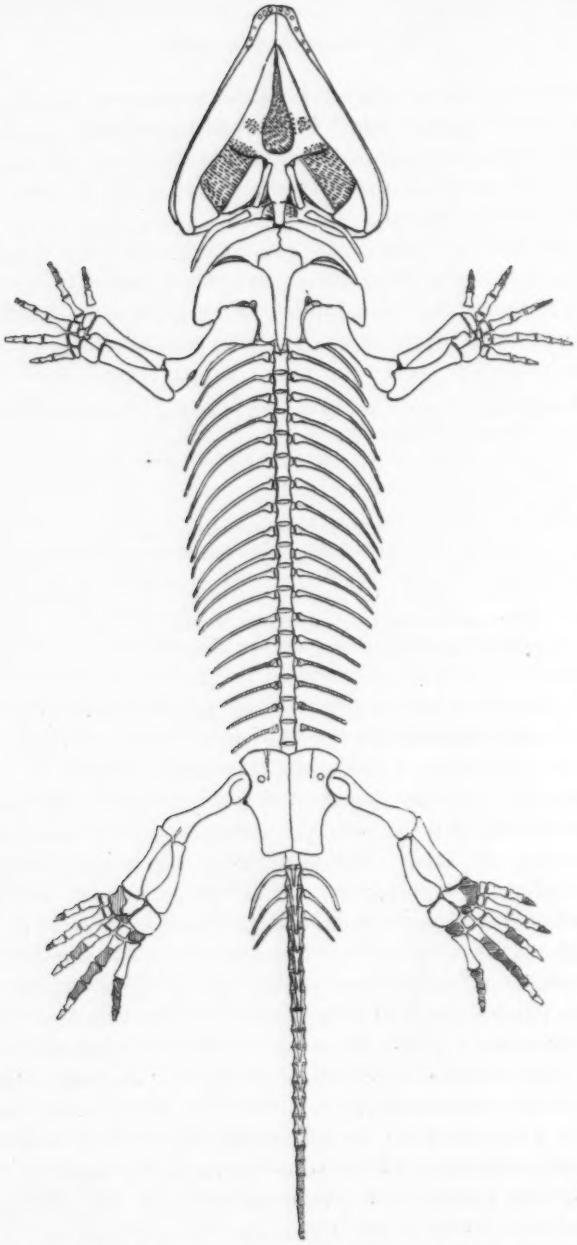

FIG. 5. *Pariotichus laticeps.* Restoration of skeleton; one half natural size.

clusion, we have only the genus *Pariotichus* left, and in all its characters, so far as they have been developed in the known species of the genus, the agreement seems sufficiently certain. Six species of *Pariotichus* have been described. Of these, *P. brachyops* is excluded by the large maxillary tooth not being below the anterior border of the orbits, but much further forward, by the relative size of the orbits, etc. *P. aguti* is easily distinguished by the elongate shape of the head, its less depressed form, etc. *P. isolomus* differs distinctly in its less expansion posteriorly, its length being distinctly greater than its width, and the absence of a posterior emargination of the cranial border. *P. incisivus* has been wrongly identified as having a single row of teeth on mandibles and maxillæ by both Case and myself, whereas Cope distinctly figures it (*Trans. Amer. Phil. Soc.*, 1886, p. 290, Pl. II., Figs. 4 and 5) as having additional teeth. The form described by me as *Labidosaurus incisivus* is therefore something else. *P. incisivus* is described and figured by Cope as having a purely reticulated sculpture of the skull, which he accepts as of specific value. Our species has the sculpture, distinctly longitudinal, on the upper side of the skull anteriorly at least. *P. aduncus* is distinguished by the size of the orbits, etc. We have, then, but a single species left, *P. ordinatus* Cope, described from such scanty material that it is doubtful if an actual comparison of the type specimen would resolve doubt as to its identity with the species herein described. Our species may, therefore, be provisionally given the name of *P. laticeps*.

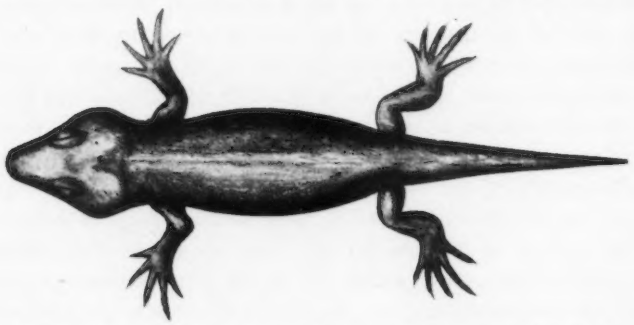

FIG. 6. Life restoration of *Pariotichus laticeps*; one fourth natural size.